FLOWERING IN *HELICONIA ROSTRATA* RUÍZ & PAVÓN

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAI'I IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

HORTICULTURE

DECEMBER 2000

BY

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IN MEMORIAM

Antonio Oliveira De Sousa

(My Father)

Because pursuing this goal I did not share his last moments

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my chairperson, Dr. Richard A. Criley for inviting me come to the University of Hawaii, his guidance, and understanding.

I very much appreciate my other committee members Dr. Kent D. Kobayashi, Dr. Robert Paull, Dr. William S. Sakai, and Dr. David D. Webb for their assistance and suggestions.

Thanks to: Dr. Osamu Kawabata for the suggestions in the statistical analysis; Dr. David D. Webb and Dr. Adelheid Kuehnle for the help with equipment and chemicals; and Mr Bob Hirano and the Lyon Arboretum for providing material of *Heliconia rostrata* used in one of the experiments.

My special thanks to Mr Ronald Matsuda and Craig Okasaki of the Magoon facility for the great help. I want to express my gratitude to faculty, staff and colleagues in the Department of Horticulture for sharing with me their skills, help, and friendship.

I will never forget the help and kindness of the friends that I meet in Hawaii, especially for the scholarly help from Derrick Agboka, Reneé and Adrian Ares, Douglas Gaskill, Michael Melzer, Javier Méndez, Monica Mejía, Teresa Restom and Mario Serracín.

I also would like to acknowledge "Universidad Centroccidental Lisandro Alvarado" and Conicit, in Venezuela, for the economic support.

Finally to my family, colleagues (in especially Norca Mogollón) and friends in Venezuela for their help, encouragement and moral support.

ABSTRACT

The factors that affect the seasonal blooming of *Heliconia rostrata* in Hawaii and how to use them to obtain off season flowering were studied. These studies show that this pattern of blooming is due to photoperiod. Competent shoots (3 or more unfurled leaves) from different experiments subjected to short days (SD) less than 11.5 hours for periods of 4 to 8 weeks did flower. Plants growing under daylength >13 hours or <12 hours but with supplemental light or night break did not flower. The critical daylength was between 11h 45m and 12h. On the other hand, night temperature did not induce flower initiation. Therefore, *H. rostrata* is a typical obligate or qualitative short day plant. The imposition of SD allows an earlier blooming season by inducing flowering, whereas the extension of daylength delays blooming by inhibiting the induction. The emergence of the inflorescence from the shoots occurred 21 to 29 weeks after the onset of SD.

Under Hawaii's natural daylength, the floral apex was observed microscopically 2 months prior to blooming. Floral shoots had from 6 to 12 leaves, depending on the number present at induction, while in non-induced shoots, the number of leaves can reach up to 15 since the apex would still produce leaves even if it had reached the competent stage to be induced.

Shoot density, daylength, and shoot generation were related with floral shoots and apex death. More inflorescences were developed in pots with one shoot per generation than in pots with all shoots per generation. At higher shoot density there were more dead shoots. The highest percentage of flowering shoots was also observed under continuous SD from all generations of shoots than in plants under 8 weeks of SD followed by

long days. The results also showed a differential response between floral and dead shoots with the generations. The second generation of shoots showed the highest flowering. The highest shoot death occurred at the first generation. Stage of development of shoots at the moment of induction and competition among shoots for assimilates were suggested as possible causes of apex shoot death.

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CHAPTER 1

INTRODUCTION

Of the more than 220 species described in the tropical Heliconiaceae (Kress et al., 1999), over three dozen species and cultivars are marketed internationally as cut-flowers (Criley, 1999). Although, heliconias are relatively new as horticultural crops, they are among the most important bold tropical cut-flowers. They are also widely cultivated as ornamental plants. Beyond their economic value, only a few heliconia species have been studied horticulturally (Criley and Broschat, 1992).

Although some heliconia species are capable of year-round blooming when their growth is not limited by environmental conditions, many other valuable horticultural species have a distinct seasonality (Criley, 1985; Criley, 1999; Criley and Lekawatana, 1990; Criley and Broschat, 1992). Studies dealing with the management of seasonally flowering heliconias have been mainly associated with photoperiod (Criley et al., 1998).

The seasonality of blooming in species with commercial interest is a critical marketing factor, and its manipulation is essential to conquer potential markets. However, blooming manipulation requires the elucidation of the mechanisms involved in flowering control. Flowering is a multistage process composed of sequences of temporal and spatial events that have specific requirements and are affected differentially by environmental, chemical, and genetic variables (Kinet, 1993).

Terminal apex death has been reported as a factor negatively affecting flower production in different heliconia species. The shoot apex death was associated in *H.*

stricta 'Dwarf Jamaican' with the abortion of the flower primordia during inflorescence development; and since high air temperature was correlated with flower abortion, Lekawatana (1995) hypotized that abscissic acid could be the promoter. However, no correlation was demonstrated. Even if the abscissic acid hypothesis has not been completely discarded, the effects of other plant growth regulators such as ethylene, and the competition among shoots for substrates could also promote abortion.

On clumping cereal and grasses plants, plant population affects initiation and growth of tillers, stem, or leaf growth, and the initiation and subsequent growth of flower initials at the shoot apex (Kirby and Faris, 1970; Bazzaz, 1997). Death of the apex was also reported in banana, a heliconia relative (Lassoudière, 1980). Since heliconias are clumping plants, shoot density could play a role in the death of their apices.

H. rostrata Ruiz & Pavon is a widely cultivated species with pendulous inflorescence of red-yellow boat-shaped bracts (Berry and Kress, 1991). This species shows a seasonal pattern of blooming from March to July in Hawaii (Criley and Broschat, 1992). Controlled studies of the flowering behavior of *H. rostrata* have not been reported. To program the flowering of this species throughout the year requires a detailed knowledge of the factors affecting flower induction and development.

Objectives

Marketing of cut flowers and potted heliconias is greatly aided by year around production which requires control of flowering. Since the factors promoting seasonal flowering of *H. rostrata* (in Hawaii March-July) were not known, and this is an important horticultural species, it was selected for study. The goal of this research was to develop a better understanding of factors influencing blooming in heliconia. The morphological and

physiological basis for flowering initiation, development, and abortion of *H. rostrata* were determined using the cultivar Five Days Peru.

The specific objectives were as follows:

I. To determine if *H. rostrata* is a photoperiodic species and, if so, to characterize the effects of daylength and temperature in its flowering.

II. To describe the physiological basis of flower abortion by analyzing the effects of daylength, number of leaves per shoot, and number of individuals in the clump during and after artificial induction or forcing of *H. rostrata*.

CHAPTER 2

LITERATURE REVIEW

2.1. Botany in Heliconia

2.1.1. Taxonomy

The most current taxonomic studies place Heliconias in the Class Liliopsida (Monocotyledonae), Superorder Zingiberanae (Scitamineae), and order Zingiberales (Kress at al., 1999). Within the order Zingiberales there are 90 genera and 2000 species, grouped in 8 families (Cannaceae, Costaceae, Heliconiaceae, Lowiaceae, Marantaceae, Musaceae, Strelitziaceae, and Zingiberaceae. Many of the species in these families are widely cultivated as ornamental plants (Kress at al., 1999).

Approximately 200 to 220 species of *Heliconia* (Kress at al., 1999), and probably at least that many forms and cultivars have been estimated in the monogeneric family Heliconiaceae (Berry and Kress, 1991). Five subgenera are currently under consideration: *Stenochlamys* revised by Andersson in 1985, *Heliconiopsis* by Kress in 1990, *Heliconia* and *Taeniostrobus* by Andersson in 1992, and *Griggsia*, created by Andersson in 1985 to group the pendulae species (Kress at al., 1999).

2.1.2. Plant characteristics

Heliconias are herbaceous sympodial rhizomatous plants that grow as clumps. Each individual of the clump is an erect shoot or stalk composed of a stem and leaves that is often terminated by a colorful inflorescence (Berry and Kress, 1991). Once a plant is mature and the environmental flower inductive factors available occur, each shoot or

tiller produces from the rhizome has the potential to generate an inflorescence. The inflorescence, with flowers in anthesis, may last from several days to several months (Stiles, 1975). When the inflorescence ceases to produce fruits, the shoot dies (Berry and Kress, 1991).

Each hapoxanthic individual is made up of an axis covered by overlapping sheathing leaf bases (hence technically called pseudostem) that can be from 0.5 m up to 10 m in length (Criley, 1985). Leaves are arranged in a two-dimensional plane (distichous), and three basic shoot habits are defined: the "musoid" type, where long petioled leaves are vertically oriented; the "zingiberoid" type with short petioles horizontally positioned; and the "cannoid" habit with short to medium-length petioles and blades held obliquely. The inflorescence has either an erect or a pendant orientation (Abalo and Morales, 1982; Berry and Kress, 1991).

The inflorescence is formed by a varying number of colorful bracts or spathes, and each bract contains varying number of flowers in a cincinnus arrangement (Berry and Kress, 1991). The inflorescence opens bract by bract, over a period of days or weeks; flowering usually starts in the older bracts before inflorescence has fully opened (Stiles, 1975). The flowers are hermaphroditic and subtended by a small floral bract or bracteole that persists through fruit development. The mature fruit is a drupe with a hard inner layer enclosing the true seed (Berry and Kress, 1991).

Heliconia leaves possess the C_3 anatomical structure and exhibit C_3 photosynthesis (He et al., 1996).

2.1.3. Ecophysiology

Heliconias are native primarily to the New World tropics, from the Ecuador to 28 degrees of latitude north and south, where most species inhabit moist or wet regions, but some are found in seasonally dry areas. They are found from sea level to 2000 m altitude. Although heliconias attain their most luxuriant vegetative growth in the humid lowland tropics at elevations below 500 meters, the greatest number of species occur at middle elevations (500 to 1,000 m). Heliconias grow best in open sites in secondary growth along roadsides, on riverbanks, and patches of light at middle-elevation rain and cloud forest habitats (Berry and Kress, 1991).

Within *Heliconia,* it is possible to find a gradient of species which are adapted to light environments ranging from rain forest understorey with extremely low daily photon flux density (PFD, μmol m^{-2} s^{-1}), species from small forest gaps, and partially open habitats along creeks, as well as the more typical species that colonize full-sun disturbance sites (Rundel et al., 1998). Large open habitats, characterized by high solar irradiance, are colonized by the species with musoid growth that form dense stands in shoots. The species with zingiberoid habit often colonize the small gaps of the forest understorey. Species such as *H. psittacorum* have a completely different habitat. In the wild, this tropical savanna species is exposed to high radiation and dry-wet periods.

The relationship between growth habitat of seven species of heliconias and their physiological and structural characteristics were studied by Rundel et al. (1998) at different light regimes in their natural forest in Costa Rica. A clear gradient was observed among the species with respect to light saturation and rates of maximum net assimilation (A_{max}). *H. latispatha*, a species from light exposed site, showed saturation at higher

photon flux density (PFD of 1400 μmol m^{-2} s^{-1}) and higher A_{max} (14-16 μmol m^{-2} s^{-1}); *H. irrasa*, a deep-shade forest understorey species showed a PFD of 250 μmol m^{-2} s^{-1} and A_{max} of 3.5 μmol m^{-2} s^{-1}. These rapidly growing herbaceous perennials appear to allocate much of their above-ground biomass to leaf tissues and have a relatively low investment in support tissues (Rundel et al., 1998).

Yellowing of fully expanded young leaves occurred in some *Heliconia* when exposed to direct full sunlight. This symptom was associated in three cultivated taxa (*H. rostrata*, *H. psittacorum* x *H. spathocircinata* 'Golden Torch' and *H. psittacorum* 'Tay') with photoinhibition by He et al. (1996). Photoinhibition describes the damage to the photosynthetic apparatus and the leaf photoprotective mechanisms that occurs in response to high light, or even moderate light, under other stresses such as temperatures, limited nitrogen supply, water, salinity, or air pollution (He at al., 1996). When grown under full sunlight, plant of all three heliconia exhibited reduced photosynthetic capacities and lowered chlorophyll content per leaf area as compared with plants grown under intermediate and deep shade. The top leaves were the most affected. Although all three taxa exhibited a decrease in photosynthetic capacity in full sunlight, the sun leaves of 'Tay' showed a higher photosynthetic capacity than those of the other two taxa. The vertical leaf angle and the lamina area of *H. psittacorum* might decrease the level of incident sunlight and therefore the leaf temperature (He at al., 1996).

Temperature limits heliconias growth. Decreasing temperature from 21 to 10 °C decreased growth and stopped flower production in *Heliconia psittacorum* (Broschat et al, 1984; Geerston, 1989). Minimum temperature, for cut flower production in this species, was suggested to be 21 °C, with increased production up to 35 °C. Although

increasing temperature will increase flowering due to an increased of the plant overall growth rate, the optimum temperature is expected to be different for every species. In addition, high temperatures have been associated with the dead of shoots which reduces flower production (Criley and Broschat, 1992). Species from tropic high altitude, may behave better at temperatures lower than the optimum reported for the low altitude *H. psittacorum*, however. Thus the selection of species and cultivars attending the environmental conditions available is key factor to ensure highest production.

Therefore, different responses to environmental conditions are expected among more than 200 species of heliconias. The growth and flower on the different species are mainly determined by specific levels of light (intensity and daylength), temperatures and water.

2.2. Horticulture

2.2.1. Status in the ornamental industry

Heliconias are mainly cultivated for cut-flowers in open fields of tropical and subtropical areas. They are also widely cultivated as landscape plants and less so as potted plants intended for interiorscape use.

From the early 1970s, small supplies of heliconias were sold at the Dutch auctions. Heliconias were listed as the only cut tropical flower besides anthuriums and remained a minor flower crop until the early 1990s (Criley, 1991; Criley and Broschat, 1992). In the past decade the unique and colorful inflorescence gave heliconias much attention among the bold tropical cut-flowers (Criley, 1990, 1998, 1999).

Heliconias are exported from Central and South America, the Caribbean and Africa countries to Europe and U.S.A. Commercial culture of this crop is also increasing

in Asia (Thailand, Malaysia, and Philippines) for exports to Japan. In the United States, Hawaii is the major producer (Criley and Broschat, 1992).

In Hawaii, the boom of heliconias as a commercial crop occurred during the 1980's. Since 1985, heliconias have been reported as a separate cut flower item by the Hawaii Department of Agriculture. Hawaii farm gate value has increased from $ 125,000 to $580,000 in 1998, produced on approximately 57 farms on several isles (NASS, 1999).

2.2.2. Research

Since heliconias are a relatively new horticultural crop with a diversity of species, varieties, and hybrids, their physiology and cultural aspects of production have not been widely studied. Research has been mainly focused on cultural aspects of small-flowered and year-round materials (Criley and Broschat, 1992). *Heliconia psittacorum* cultivars and the hybrid (*H. psittacorum x H. spathocircinata* 'Golden Torch') have received the most attention (Broschat and Donselman, 1983 a, b; Broschat et al., 1984; Catley and Brooking, 1996; Clemens and Morton, 1999; Geertsen, 1989; Manarangi et al., 1988; van Raalte and van Raalte-Wichers, 1973). Other well-studied small species are the seasonal *H. stricta* 'Dwarf Jamaican' (Criley, 1982; Criley and Kawabata, 1986; Lekawatana, 1986; Lekawatana, 1995; Lekawatana and Criley, 1989), and *H. angusta* (Kwon, 1992; Lekawatana, 1986; Sakai et al., 1990 a; Sakai et al., 1990 b). Less work, has been done on the large-flowered species (Criley and Lekawatana, 1990, 1995; Criley and Sakai, 1997; Maciel, 1991; Maciel and Rojas, 1994).

In their habitats, the seasonal flowering of some Central American species was attributed to the dry–wet cycle (Stiles, 1975). Nevertheless, normal rainfall patterns in Hawaii differ from those in Central America and seasonality is still observed, even under

irrigated conditions. These observations led Criley (1985) to suggest that other environmental factors might be involved in the blooming process.

The management of seasonal flowering in heliconias has been focused on photoperiod, and these studies have been mainly done at the University of Hawaii. Since heliconia photoresponse was reported for the first time by Criley (1982), in *H. stricta* 'Dwarf Jamaican' (then called *H. humilis*), a few species have been recognized for their sensitivity to photoperiod (Criley et al., 1998). Some of them have short-day (SD) response, while others are long-day (LD) (Criley and Kawabata, 1986; Lekawatana, 1986; Criley and Lekawatana, 1990; Geerstsen, 1990; Sakai et al, 1990a; Kwon, 1992; Criley and Sakai, 1997).

Plants species frequently selected to study flowering induction and development are sensitive to a single inductive cycle (i.e. appropriate daylength to cause flowering). Whereas simple model systems are always attractive to the investigator, simple requirements may not be typical of flowering plants. Also, experimental materials are selected for the facility to examine the reproductive structures, since the changes occurring in these structures can be normally observed with nothing more elaborate than a hand-held magnifying lens (Attridge, 1990). Unfortunatly, heliconias do not full either one of these ideal characteristics, long periods under inductive conditions and later development are required, and their hidden apex inside the pseudostem makes study difficult. In addition, their large size, relatively slow development, and growth habit as clumping plants complicate their handling and variety of responses.

2.3. Flowering

During the transition from vegetative to floral, the whole meristem acquires a new developmental fate. The acquisition of the new developmental fate is associated with the formation of the inflorescences and flowers. This is a sequential set of changes that occurs throughout the plant and forms an interactive network (McDaniel, et al., 1992).

Although flowering is an integrated process it can be divided, from the physiological perspective, into at least two major phases: floral initiation and subsequent flower development (Bernier, 1988). These phases of the flowering process may have different requirements. Some plants are completely dependent upon environmental signals, whereas others rely on internal developmental cues. In *Arabidopsis* and *Antirrhinum* there is support for both pathways of floral induction. Much less is known about genes controlling the flowering process in monocotyledonous plants (Colasanti et al., 1998; Kyozuka et al., 1998). The relationship between floral bud formation and floral gene expression were reported recently in apple (Kotoda et al., 2000)

The floral initiation requires vegetative apices producing leaf primordia to be transformed into flowering apices producing flower primordia (Attridge, 1990). If one focuses on the initiation of a floral meristem, two developmental states are acquired and expressed in order for a meristem to initiate morphogenesis. First, the meristem cells become competent to respond to the developmental signal that evokes or elicit a florally determinate state. Second, the meristem is committed into a florally determined state and then expresses that state (McDaniel, 1994). Thus, floral initiation results in changes in the apex after it has been induced and evoked to become florally determined.

Numerous patterns of morphological change follow evocation. Of interest within the context of floral initiation is the observation that during the transition from vegetative to floral development, the patterns of cell division and the organization of the meristem exhibit marked changes (McDaniel, 1994). Cells, organized as a shoot apical meristem, form lateral organs in specific spatial patterns and temporal sequences. A vegetative meristem expresses one pattern and sequence, while a floral meristem expresses a different pattern and sequence.

After plants have been exposed to flowering inductive conditions for a sufficient length of time, they become committed to flowering. Even though no visible change may have occurred at the apex, biochemical and cellular changes have already occurred. Morphological growth changes, such as meristem form, leaf primordia, apical internodes, and axillary meristems are only observed later. In many perennial plants grown in seasonal environments, the developmental commitment of meristems to vegetative or reproductive organs takes place months or years before the organs are elaborated, and may be precede by a considerable amount of time the investment of resources to build the organs (Geber at al., 1997).

Vegetative growth of plants exhibiting monopodial developmemt, is limited by the timing of the conversion of the shoot apical meristem to inflorescence development. Once an inflorescence is produced by the shoot meristem, no more leaves can be added to the shoot and it becomes determinate. Thus, the length of vegetative growth is determined by how early or late flowering begins (Irish, 1998).

Floral initiation is usually correlated with stem elongation in many grasses. In barley, floral initiation and some floral development of the shoot apex precede stem

elongation, and it has been proposed that stem elongation depends upon some hormonal stimulus from the floral apex (Nicholls and May, 1964a,b). However, there are cases in the grasses when this sequence is not obligatory and the stem elongation can occur before or without floral initiation. Thus flower initiation and floral development are two separate phenomena, each with its own control system (Kirby and Faris, 1970).

2.4. Factors affecting flowering

Plants have evolved intricate schemes to coordinate the transition to flowering with optimal environmental conditions and developmental states. Some plants are completely dependent upon environmental signals to evoke flowering, whereas others rely on internal developmental cues perhaps correlated with plant size to signal the time of transition (Lang, 1965; Bernier,1988; MacDaniel et al., 1996). Most plants integrate both environmental and developmental signals to elicit flowering.

The factors that cause flower initiation in plants and their effectiveness are variable. Although most extensive studies have concentrated on the effect of photoperiod in the determination of flowering time, other factors, such as growth temperature, vernalization, nutrient availability or hormones can be equally important under certain circumstances. For example, in SD *Pharbitis*, flowering can be induced under LD by poor nutrition, high irradiance, low temperature, root removal, and application of a cytokinin, among others. There is an ever-increasing list of plants, originally considered to be strictly photoperiodic, that can also be made to produce flower buds by a variety of other factors besides daylength. As a rule however, flowering in response to these alternate factors is relatively weak (Zeevaart, 1976).

In sorghum, for example, the interaction of environmental and endogenous factors in flower initiation is well known (Quinby et al., 1973). The development of this crop is delayed by an increase in photoperiod above a critical value. The four maturity genes that control time of floral initiation and duration of growth are influenced by temperature (Quinby et al., 1973).

2.4.1. Environmental factors

Among environmental influences, light is one of the most important factors determining time and magnitude of flowering, as well as the rate and direction of flower development.

Light intensity

The minimum radiant energy requirement for flower initiation varies widely among species. High irradiation is associated with increased flower production in most self-inductive species in which flowers are initiated autonomously in every growing shoot after a certain number of leaves have been formed (Halevy, 1987). In the case of roses, low irradiance causes a reduction in flowering mainly because of an increase in flower abortion (Halevy, 1987). This response has generally been attributed to the effect of light on photosynthesis. However, light has also shown to increase sink strength of rose flower buds (Zieslin and Halevy, 1975).

In their natural habitats, heliconias from middle elevations grow best in forest clearings, with number of flowering stalks decreasing as light intensity decreases (Stiles, 1975). In the group of *H. psittacorum*, insufficient light intensity is a factor limiting commercial flower production (Broschat and Donselman, 1983a; Broschat et al, 1984; Manarangi et al., 1988). When grown in full sun light, the cultivar Andromeda produced

three to four times more flowers than plants under 63 % shade (Broschat and Donselman. 1983a). Reduced light intensities within beds due to mutual shading eventually limits flower production in this species, even under full sun light (Broschat et al, 1984). In *H. bihai* and *H. latispatha,* cultivated under three shade levels (0, 40 and 60 %), the number of shoots was higher under full sun light conditions for both species (Maciel and Rojas, 1994). Thus the higher inflorescence production in heliconias seems to be mainly associated with an increased number of stems and growth of whole plants under the higher light intensity.

Daylength

In many species, daylength is the controlling factor for floral initiation (Kinet, 1993). Many of the plants whose flowering physiology has been well characterized have a strong response to photoperiod. Photoperiodic classification of plants has nothing to do with the particular daylength at which the plant flowers, but it rather depends on whether flowering is promoted when the daylength is increased or decreased. There are a number of photoperiodic response types. Nevertheless, the basic types (SD and LD plants) have been the most thoroughly characterized (Bernier et al., 1981a,b; Thomas and Vince-Prue, 1997; Vince-Prue, 1975).

In most photoperiodic response types, there are plants with an absolute response and others with a quantitative one. Thus, plants referred to as absolute or qualitative SD or LD has an obligate requirement for SD or LD, respectively. These plants are characterized by an abrupt change in behavior over a narrow range of daylength and consequently have a sharp "critical daylength". On the other hand, quantitative or facultative SD or LD will produce flower buds under any daylength but will do it earlier

in SD or in LD, respectively. Such plants may or may not have a clear-cut critical daylength. The concept of critical daylength is not without difficulties. Even for absolute photoperiodic species, the critical daylength can be profoundly modified by various environmental parameters, e.g. nutrition, temperature, light flux, plant age, etc. A factor influencing the critical daylength is the number of favorable cycles given (Bernier et al., 1981a,b).

Photoperiodism requires the participation of both a photoreceptor, generally thought to be a phytochrome (which distinguishes light and darkness), and a time measurement process that appears to be based on an endogenous clock. Flowering was found to exhibit a rhythmic response as a function of the total cycle length in SDPs by exposing plants to a SD followed by dark periods of increasing length. The period of this rhythm was about 24 h, suggesting the involvement of an endogenous circadian clock. A similar kind of response was observed in LDP. In SDP's at least three distinct photoreactions are relevant to photoperiodic regulation of flowering, and a phytochrome appears to be the photoreceptor. The perception of the daylength signal occurs primarily in the leaves (Thomas and Vince-Prue, 1997). It is also generally accepted that leaves are the organs that measure the photoperiod. Leaves then send a signal (s) that results in the initiation of floral development by the shoot apical and lateral (axillary) meristems (Lang, 1965; Vince-Prue, 1975).

Flowering will often occur when plants are returned to non-inductive cycles after a sufficient number of favorable photoperiodic cycles. In the most extreme cases, a single favorable (inductive) cycle is able to bring about floral initiation. At the other extreme, favorable cycles must be continued until the apex has become recognizably floral. In

Xanthium, for example, the magnitude of flowering response increases with an increase in the number of inductive cycles; this effect seems to be associated with the leaves rather than with the apex. All leaves are not necessarily equally sensitive to induction. Also, it is possible that a true 'induced state' does not develop and that each cycle produces some stimulus. (Thomas and Vince-Prue, 1997).

Sharp critical photoperiods close to 12 hr have been observed in certain varities of sugar cane and rice. It is obvious that when such plants are grown at low latitudes, they are able to perceive small changes in the daylength and exhibit seasonal flowering. Although sugar cane required an intermediate photoperiod around 12.5 hr for floral initiation, further development and elongation of the inflorescence proceeded under shorter photoperiods (Zeevaart, 1976). In Hawaii, the floral induction of commercial sugar cane hybrids normally occurs during the first 20 days of September. A minimum of 10 inductive days is required for flowering to occur, while 15 or more inductive days are required for maximum flowering. Sunrise to sunset during September 1 to through September 20 ranges from 12 hr 33 min to 12 hr 10 min, providing a sequence of lengthening nights which are more stimulatory to flowering than are shortening nights (Moore and Heinz, 1971). In several short-day rice cultivars, however, the flowering is reduced when the day is extremely short or when the light period is given at a low irradiance (Ikeda, 1985).

On the other hand, night interruption will prevent flowering in many SDPs under inductive conditions (Thomas and Vince-Prue, 1997). SDPs often show an all-or-none response to a night break. Night interruption or night-break involves interrupting dark period with a period of lighting (Thomas and Vince-Prue, 1997). A few minutes of night-

break can be enough to prevent flowering in most SDPs, but chrysanthemum requires several hours from incandescent lamps (Cathey, 1969). The night-break response in SDPs requires low energy. However it differs with the leaf optical properties of the species (Cockshull, 1984). The effectiveness of night-break is also dependent on the point in the cycle when the treatment is given (O'Neill, 1992). The relationship between the time of sensitivity to a night break is not a simple one; in some plants, such as *Lolium temulentum* and *Coleus*, light had the greatest effect during the middle of the night period, while in others (*Chrysanthemun, Glycine, Pharbithis* and *Xanthium*), a period of great sensitivity to light exists about 7 to 8 hours after the onset of darkness (Thomas and Vince-Prue, 1997). Thus, it is unwise to assume, as is often done for commercial regulation of flowering that the 'best' time to give at night-break is a midnight (Thomas and Vince-Prue, 1997).

The amount of light necessary to saturate the night-break inhibition of flowering varies with species, conditions and the time of exposure (Lumsden and Furuya, 1986). The night-break inhibition of flowering in SDP is clearly dependent on PFr; thus phytochrome is associated directly with the photoperiodic mechanism. There is also evidence that leaves and/or roots are sources of signal(s) inhibitory to the initiation of flowering (Thomas and Vince-Prue, 1997).

Heliconia species such as *H. psittacorum*, *H. hirsuta*, *H. chartacea*, *H. episcopalis, H. nikeriensis, H. indica, H. solomonensis,* and some cultivars of *H. stricta* and *H. bihai* among others show year round flowering under suitable environmental conditions for growth. These are considered as daylength neutral species. However, when grown under extreme conditions, such as low or high temperatures and high latitudes,

these species could show a seasonal flowering as a consequence of their effects reducing in the overall growth or inducing dead of shoots.

Among the photoperiodically studied species, *H. stricta* 'Dwarf Jamaican', *H. wagneriana*, and *H. aurantiaca* are SD plants. *H. stricta* 'Dwarf Jamaican' is a small plant that requires at least 4 weeks under inductive daylengths of 8 or 9 hours (Criley and Kawabata, 1986; Lekawatana, 1986). The minimal number of inductive weeks required to flower was not reported for *H. wagneriana* and *H. aurantiaca* (Criley and Sakai, 1997; Geerstsen, 1990). In *H. angusta*, a long day plant, at least 7 weeks with a minimum of 13 hours of daylength was required for flower induction. However, more plants flowered following 8 and 9 weeks of treatment than at 7 weeks (Lekawatana, 1986; Sakai et al, 1990a; Kwon, 1992). The critical daylengths have not been determined for *H. stricta*, *H. wagneriana* and *H. aurantiaca.*

No photoperiodic effects on flower development were determined in *H. stricta* and *H. angusta*, since differences in the daylength had no effect on flowering time or number of spathes per inflorescence (Lekawatana, 1986 and 1995; Know, 1992).

Temperature

Considerable variation exists among species in the extent to which temperature markedly influences the critical night length (Thomas and Vince-Prue, 1997). For example, strawberry is strictly photoperiodic only at temperatures above 15 °C (Guttridge, 1985).

Temperature changes are known to interact with photoperiodic processes by shifting the phase and amplitude, thus initiating rhythmic responses in plants (Searly, 1965). Even small changes in temperature may suffice to alter the plant response

(Bernier, 1988). In chrysanthemum, high night temperature at the start of the inductive SD delays the onset of flower initiation (Cockshull and Kofranek, 1994). On the other hand, it is well known that flower initiation is promoted by low temperature in a number of species.

Since floral initials differentiate during low temperatures, the direct effect of low temperature on flower initiation is distinct from vernalisation. Vernalisation is an inductive phenomenon that is only completed when the plant is returned to higher temperature and, in many cases, to particular photoperiodic regimes (Thomas and Vince-Prue, 1997).

Other than daylength, temperature may play a role in heliconia flower induction and flower development. While no heliconia species have been reported to start flower bud initiation in response to a temperature stimulus, suitable temperatures affect the flowering, due to the overall growth rate of the plants. However, when night temperature is increased from 15 °C to 25 °C during the initial stimulus period (4 weeks of SD) in *H. stricta* 'Dwarf Jamaican', the percentage of reproductive pseudostems decreased from 53% to 14 % (Lekawatana, 1986).

2.4.2 Endogenous factors

Plants that show quantitative photoperiodic responses eventually flower without exposure to inductive cycles. Similarly, day-neutral plants flower in the absence of photoperiodic induction. In both cases, the age of the plant appears to be a major factor in the attainment of reproductive growth (Thomas and Vince-Prue, 1997). Presumably, this is a mechanism to ensure that individuals accumulate sufficient resources to allow successful reproduction (Reekie, 1997).

Allocation of resources to reproduction in many plant species begins only after plants have attained a certain mass, size, or age. It has been also assumed that in many plants, attainment of a certain size, rather than age, is the critical factor for reproduction (Bazzaz, 1997). Mass and size, especially for perennials, while generally correlated within species, may have completely different relationships for different species or different environments. For example, in the herbs solidago and aster, a minimum size (mass) threshold for sexual reproduction is required (Schmid et al., 1995). However, there appears to be no minimum mass for clonal expansion, which is critical for occupation of more habitats, and for habitat selection (Bazzaz, 1997).

Plants are unable to respond to the appropriate photoperiod when still in the juvenile stage. The juvenile stage may last from a few days in herbaceous plants to a few years in woody species. Evidence exists to support both the hypothesis that there is a plant-apex signal which brings about transition to the mature phase and vice versa (Attridge, 1990).

Older plants are more easily induced to flower (Thomas and Vince-Prue, 1997). In SD *Amaranthus* species, the greater inductiveness is expressed by a reduction in the number of leaves produced by the shoot before its transformation into the reproductive state (final leaf number) (Kigel and Rubin, 1985). In the SDP *Euphorbia pulcherrima*, the time of initiation under LD appeared to be a function of the ontogenic age of the meristem, since leaf-removal did not affect the leaf number at flowering (Evans et al., 1992). Another possibility is that inhibitory factors from roots decrease with the age, since the distance between roots and the apex increases as the plants continue to grow (Thomas and Vince-Prue, 1997).

Many monocarpic plants must reach a minimum critical size before reproduction can be induced. A direct consequence of this requirement is that time of reproduction in monocarpic perennials is more closely correlated to size than age. Therefore, any environmental factor that affects growth rate will also affect time to reproduction (Reekie, 1997). In SD rice plants, the degree of photoperiod sensitivity has been reported to vary with age. However, this aging effect is probably the result of other factors such as seedling vigor (Yin et al., 1997).

Rice cultivars do not respond to photoperiod during the entire period from sowing to flowering (Vergara and Chang, 1985). While the durations of the pre- and post-inductive phases are unaffected by photoperiod, that of the inductive phase is governed by photoperiod and persists longer in less-inductive regimens. Therefore, since rice is a short-day plant, long days increase the length of inductive phase (Roberts and Summerfield, 1987). Unlike photoperiod, which affects only a limited period in the crop life cycle, temperature modulates all successive stages of development (Roberts and Summerfield, 1987).

Photoperiod and temperature are the two environmental variables also known to affect maize development through their influence on leaf number (Tollenar and Hunter, 1983). The hybrid Guelph GX122 showed that after a juvenile phase, during which leaf number of plants is not affected by photoperiod and temperature, a phase occurs where plants are sensitive to photoperiod and temperature. Following this phase, the plants show a short insensitive phase again, just prior to tassel initiation. The transition from vegetative to reproductive development (i.e. tassel initiation) occurred at the leaf stage that is numerically equal to 50 % of final leaf number (Tollenar and Hunter, 1983). In this

crop, defoliation has been used to modify the flowering date and reduce tillering (Croskston and Hicks, 1978; Crockett et al., 1978).

In banana, a self inductive plant relative to heliconia, flower initiation could be triggered once a specific amount of leaf area has been produced (Stover and Simonds, 1987). Defoliation can also be used to delay banana inflorescence differentiation (Turner and Hunt, 1987). The delay caused by defoliation depended on the severity of the defoliation and the stage of growth at which it occurs. Defoliation also delays the start of the sucker growth on the parent, then delays the harvest date of the shoot in the following generation. However, when defoliation is applied to the mother plant at the emergence of the inflorescence, ratoon growth was accelerated (Stover and Simmonds, 1987; Robinson et al., 1992).

Juvenility has not been reported in heliconias, although it probably exists in the seedling stage. Since cultivated heliconias are mainly propagated vegetatively by rhizomes (Criley, 1986 and 1988), their flowering begins after the plant achieves sufficient size to support the energy requirements of reproduction (Criley and Broschat, 1992). In the SD *H. stricta*, the expanded leaf count at the start of inductive daylength was shown to be an important guide to the capacity of the plant to respond to stimulus (Criley and Kawabata, 1986). At least three leaves per shoot are required for induction in this species (Lekawatana, 1986; Lekawatana and Criley, 1989). A similar number is also required by the LD *H. angusta* (Kwon, 1992). In *H. chartacea*, a minimum of four expanded leaves was estimated (Criley and Lekawatana, 1995). In *H. bihai* the minimum number of leaves was found to vary according to the shade levels and shoot generation (Maciel, 1991; Maciel and Rojas, 1994). Shoot generation was also a factor affecting the

final leaf number in the flower shoots of the day-neutral *H.* 'Golden Torch' cultivated in pots under controlled conditions; where the number of leaves decreased with the generation (Catley and Brooking, 1996).

2.4.3. Factors promoting abortion

Assimilate allocation

The young developing flower bud is a major assimilates sink. The flower bud is a weaker sink compared with the vegetative apices under stress conditions such as inadequate supply of assimilates (Halevy, 1987). Environmental stresses caused by light, temperature, and water promoted abortion, blasting, or abscission in the developing flower bud while other organs are only slightly affected (Kinet and Sachs, 1984; Shilo and Halevy, 1976 a,b,c; Halevy, 1987). Recent experiments with pea suggest a carbon metabolism role in the process that triggers abortion on a specific phytomere position of the stem. These changes in carbon metabolism have been linked to a reduction in photosynthesis and an increase in respiration, and could be attributed to mild stress caused by high temperature (Guilioni et al., 1997).

In some plants, assimilate allocation to the reproductive organs (e.g., flowers) may exceed the ability of the plants to mature at all because of resource limitations. Overproduction of flowers and subsequent abortion has been observed in many plants. In these cases abortion can be a mechanism for adjusting reproductive output to the level of resources available in the particular habitat (Bazzaz, 1997).

The switch from vegetative to reproductive in many plant species is gradual rather than abrupt, and plants differ in their mixed allocation phase where both vegetative and reproduction take place simultaneously. Thus a challenge to allocation theory is presented

by clonal plants, because they produce new individuals both sexually, through the seed, and asexually by elaborating eventually independent individuals (Bazzaz, 1997). Also, because phenological events in plants are dependent on environmental variation, the length of this mixed allocation cycle is also variable and dependent on environmental circumstances (Bazzaz, 1997).

Besides vegetative growth and reproductive growth are usually regarded as antagonists, many studies on growth and productivity at the community and ecosystem levels assume that vegetative growth is strongly coupled with reproductive allocation (Bazzaz, 1997). The relationship between these two forms of reproduction is not well understood. Connections between daughter ramets and their usually larger parents may remain for a long time in some species or only for a short time in others. The kind and the quantity of resources translocated to daughter ramets (and vice versa) are not fully known, but they are likely to involve sugars, amino acids, water, and nutrients (Bazzaz, 1997).

It has been demostrated in different grass and cereal species that mutual exchange of metabolites occurs between parent and daughter tillers and between sister tillers (Marshall and Sgar, 1968; St-Pierre and Wright, 1972). At early stages of development, the whole aerial part of a grass plant is an integrated physiological unit. However, as each tiller grows, the amount of metabolite transferred between adjacent shoots declines and larger tillers tend to become independent (Marshall and Sgar, 1968). The change from an interdependent to a largely independent state has been recorded using autoradiography. For example, the main shoot and tillers of the Italian ryegrass (*Lolium multiflorum)* differed in the extent of their carbon exchange and in their degree of independence. The

main shoot has the major role in the plant system, presumably because it is both a well established source (in the support of daughter tillers) and an important sink (in the formation of future tillers). While the main shoot was the most interdependent, the oldest daughter tiller of the main shoot is the most independent (Marshall and Sgar, 1968). The existence of a reciprocal exchange of assimilates between tillers means that each must be simultaneously both a source and a sink (Marshall and Sgar, 1968). However, St-Pierre and Wright (1972) reported that the tillers of Timothy grass (*Phleum pratense*) under stress behaves as separate units without supporting the main shoots. Obviously, different species behave differently.

On the other hand, shading can be a major source of stress for many crop species by reducing photosynthate. In crops as diverse as wheat, cotton, soybean, pepper, and tomato, reproductive allocation is determined, among other things, by early abortion of flowers and young fruits (Ballaré et al., 1995).

Plant density

In crops grown at dense populations, shading is mostly caused by neighboring plants of similar size and genotype (Ballaré et al., 1995). Plants grown in populations are morphologically and functionally different from plants grown as isolated individuals. This plastic adjustment is triggered in part by mechanisms that use information about the canopy light environment, transduced by specific photoreceptors to promote alternative developmental programs. Increased height, altered shoot direction and altered branching and assimilate allocation to reproductive structures and vegetative storage organs (e.g. bulbs and roots) are among the responses to crowding (Ballaré et al., 1995). Within plant canopies, the light environment is characterized by low levels of blue and red light (the

visible wavelengths that are most absorved by chlorophylls) and high levels of far red (Ballaré et al., 1995). Changes in red:far-red ratio are used by plants to sense the proximity of neighboring individuals. The decrease in the red:far-red ratio caused by neighboring plants can reduce branching (tillering) rate even if the production of new branches is not limited by the availability of PAR in open grass canopies (Ballaré et al., 1995).

Since cereals tend to produce more tillers than they can support depression of tillering (induced, for example, by low red:far-red ratios) may be beneficial, because it would reduce the number of secondary and tertiary tillers that usually depress the harvest index by increasing the number of dead and/or sterile flowers. Tillering is influenced by other environmental factors besides light, such as nutrition and atmospheric composition (Ballaré et al., 1995; Casal, 1988).

In rice, tillering is altered by environmental and cultural variables such as light and planting density (Volk and Mitchell, 1995). Since under conditions of increased photosynthesis, a day-neutral cultivar partitions preferentially to leaves and tillers, Fagade and De-Datta (1971) found that above a certain number of tillers, developing rice panicles compete for photosynthates with newly developing vegetative tissue. The larger number of tillers forming under conditions of continuous light is offset by the fact that fewer of them bear fertile panicles (Volk and Mitchell, 1995). Tillers that formed later in development are smaller and have a longer maturation time than the earlier ones.

In banana, Lassoudiere (1980) reported that when all suckers are allowed to grow, only two of them flower while the rest die. Selection of a sucker follower, by removing the extra ones, is a horticultural practice aimed to synchronize and promote banana

production. In *H.* 'Golden Torch', plant competition is expect to be as one of the factors affecting flower production (Clemens and Morton, 1999). Since heliconias are naturally clumping plants, the competition for available assimilates between the pseudostems in the clump could affect flower development and possible abortion.

Environmental factors

High irradiation has been associated with increased flower count in most self-inductive plants such as rose, where flowers are initiated after a certain number of leaves have been formed (Halevy, 1987). The low irradiance causes a reduction in flowering mainly because of an increase in flower abortion. Even when this response has been attributed to the effect of light on photosynthesis, light has also shown to increase sink strength of rose flower buds (Zieslin and Halevy, 1975)

The factors responsible for inducing abortion in heliconia may be similar to those mentioned above for other sensitive plants where factors such as low light intensity may be involved. Even so, no differences are reported for abortion in *H. stricta* 'Dwarf Jamaican' grown under outdoor conditions of light intensity (full sunlight, 40% sunlight, and 20 % sunlight) after a floral initial stimulus of 4 SD weeks. Thus light intensity seems not to be a factor in the induction of abortion. But, this species shows differences among plants growing at different pre and post induction daylength (under LD and continuous SD). Abortion is not found in plants under continuous SD. Plants that develop 3 and 4 leaves prior to the induction period (4 SD weeks) showed 54 and 77% abortion, respectively (Lekawatana, 1995). However, this experiment was not designed to separate possible cause of abortion, as a function of daylength prior to flower induction from the daylength postinduction.

Death of the shoot apex has been reported in potted plants of *H. stricta, H. angusta* and *H. wagneriana* when forced to flower under artificial inductive daylengths (Criley and Kawabata, 1986; Lekawatana, 1986; Criley and Lekawatana, 1990; Lekawatana, 1995). Also, death has been reported for *H. chartacea* (Criley and Lekawatana, 1995), *H. bihai* and *H. latispatha* (Maciel, 1991) cultivated under natural field inductive conditions. Higher values of abortion, above 50%, have been reported elsewhere (Lekawatana, 1995).

The abortion in *H. stricta* 'Dwarf Jamaican', growing under different night temperatures (15, 20 and 25°C) during the period of induction, is 20% for all treatments (Lekawatana, 1986). But in a subsequent experiment, this species shows differences in the percentage of flower bud abortion during the inflorescence development at temperature treatments of 18, 21, 24, and 28°C. Increasing night temperature from 18 to 28°C increases the percentage of aborted flower buds from 0% to 19.2%. These results suggest a critical effect of temperature, promoting abortion during the inflorescence development period. However, it is unknown if it is a direct or an indirect effect.

Plant growth regulators

The shoot apex death, in *H. stricta* 'Dwarf Jamaican', was associated with the abortion of the floral primordia during inflorescence development (Lekawatana, 1995). Since high air temperature are correlated with flower abortion, Lekawatana (1995) hypothesized that abscissic acid (ABA) could be the promoter. However, no correlation are demonstrated in his study which dealt only with ABA in the foliage and not at the shoot apex. Even though the ABA hypothesis has not been completely discarded, the effects of other plant growth regulators such as ethylene and the competition among

shoots for substrates need to be addressed as factors promoting the abortion. So far, ABA has been the only plant regulator studied by a well-supported analysis in heliconia inflorescence abortion.

The role of ABA, as a promoter of flower abortion, is not fully understood. Increasing ABA levels parallel an increase in ethylene production (Abeles, et al., 1992). ABA has been shown to promote ethylene production by leaves, explants, flowers, fruits, and cultured buds. The ability of ABA to increase ethylene production is correlated with the increase in flower abscission. These observations suggest that ABA increases ethylene, and the higher ethylene causes flower bud abscission (Abeles, et al., 1992).

Changes in either ethylene content or sensitivity to ethylene as a result of changes in environmental conditions such as light, temperature and humidity may be a reason for the heliconia bud abortion. Ethylene is directly involved in the promotion of flower bud abortion or blasting in many plants (Halevy, 1985). For instance, greenhouse production of lilies during the winter season is hampered by long periods of low light that induces ethylene stress production and the abscission of developing flower buds. Shortening daylength period leads to an increase in ethylene production from the buds (van Meeteren and de Proft, 1982). Irradiance has been shown to influence ethylene evolution in numerous plant systems (Abeles et al., 1992). Plants exposed to supraoptimal temperatures can produce ethylene. The failure of several cultivars of begonia to develop flowers at high temperatures during the summer season was associated with ethylene (Mekers et al., 1983). The optimum air temperature for ethylene production is close to 30 °C. Ethylene usually reaches a maximum rate of production at 35 °C, declining above this value and ceasing at around 40 °C (Abeles et al., 1992).

The rate of ethylene production varies at different stages of growth and development, and the levels also vary with the organ. Small increases in ethylene production, of the order of two- to three fold, have been noted during periods of flower bud formation and development (Abeles et al., 1992). Due to the heliconia apex location inside of the leaf sheaths, small changes in ethylene could be detrimental during early phases of flower development. It may be more important to consider the inflorescence characteristics, where the differentiation of flowers occurs over time, and the levels of ethylene may increase inside the cavity where the apex is located.

While usually considered to be a promoter of senescence and an inhibitor of growth and elongation, ethylene can promote flowering and stimulate cell elongation in certain plants or tissues. Ethylene-induced flower is limited only to a few groups of plants; the best known are in the family Bromeliaceae (Abeles, 1973).

Various chemicals, in particular plant growth substances, have been applied to plants under non-inductive conditions to test whether these compounds cause flower formation (Zeevaart, 1978). Cytokinins and ethylene have been reported as promoting or inhibiting flower initiation in a variety of plants (Bernier et al., 1981a; Abeles et al., 1992). Cytokinins are not used to enhance flowering directly in flower crops. Their only practical use in the control of flowering crops is the reduction of flower bud abscission in a few cut flowers and in extending post-harvest life of cut flowers and foliage (Halevy,1985; Abeles et al., 1992).

The relationship between flower formation and stem elongation has been of concern to physiologists for a long time (Zeevaart, 1976). Gibberellins are considered to play a major role in both processes; they have been implicated in the stem elongation

(bolting) that often follows floral initiation and in the induction/initiation processes per se. Intriguingly, GAs structures and doses that are highly florigenic often have nil or minimal effect on stem elongation. Conversely, for GAs known to be "effectors" of stem elongation, very high doses are often required to obtain an optimal flowering response (Pharis, 1991). LD treatment with exogenous GA under SD conditions showed that stem elongation occurred before floral development, whereas in normal flowering under LD, both flowering and stem elongation appear to occur simultaneously.

The use of plant growth regulators (PGRs) for the manipulation of growth and development in ornamentals is more common than in most other commercial crops. The most widely used PGR in ornamentals are the growth retarding chemicals (Halevy, 1985). However, their use in heliconias has been limited. Growth retardants have been experimentally used for height control in pot production (Tjia and Jierwiriyapant, 1988; Criley and Lekawatana, 1988). Lekawatana and Criley (1989), also reported the effect of growth retardants such as flurprimidol inhibiting inflorescence development. Cytokinin has also been used to break bud dormancy when applied to the rhizome (Criley, 1995). The synthetic tertiary amine bioregulator DCPTA, has been reported to have potential to cause earlier flowering and increase crop productivity in some cut-flower crops. I was reported enhances growth and flowering in *H. caribaea* and *H. stricta* 'Dwarf Jamaican' (Broschat and Svenson, 1994). In a commercial nursery in Florida, early flowering of *H. angusta,* a LD species, growing in pots was observed after gibberellic acid application (Ball, 1988). Unfortunately, this experiment did not follow scientific methods.

CHAPTER 3
THE TRANSITION TO FLOWERING IN *H. ROSTRATA* RUÍZ & PAVÓN. MACROMORPHOLOGICAL AND ANATOMICAL CHANGES AT THE SHOOT APEX

3.1. Abstract

Heliconia rostrata Ruiz & Pavón shows seasonal blooming with inflorescence emergence starting from late February to June in Hawaii. This blooming pattern is related with photoperiod in this chapter. Inflorescence initiation and development occurred without any external evidence of these processes until the inflorescence emerged from the pseudostem. Microscopic observations of dissected apices indicated that the plant was still vegetative at least until December. The minimum number of expanded leaves found in shoots at the reproductive stage was three. By early January, plants that had four or more unfurled leaves already developed two to five bracts on the inflorescence apex. The number of leaves subtending the inflorescence in the dissected flowering shoots varied from 5 to 12. The leaf number depended upon the time between shoot emergence and flowering stimulus. Shoots that reached three unfurled leaves by January had fewer leaves at flowering stage.

The morphological changes of the terminal shoot apex from vegetative to flowering stage (transition) are described. The anatomical sections reveal that the apex in the vegetative phase was domed and a maximum of four furled leaves, including one leaf primordium, were observed surrounding it. The growth of the leaf primordium was highly synchronized with the growth of the most recently formed leaves. Along with the

transition to inflorescence development, more primordia were observed on the apex, which ultimately give rise to the bracts. Except for the first sterile bract, a cincinnus primordium (flower cluster) was detectable in a bract axil when the next bract began to develop. Flower differentiation on the cincinnus began when many bracts were well-developed.

The reproductive plant status was easier to detect under the microscope when the inflorescence had at least three bracts than earlier. The increase in longitudinal height of the internodes was among the first detectable morphological changes in the apex.

3.2. Introduction

Heliconias are herbaceous and rhizomatous plants that grow as clumps, with each individual monoecious and hapoxanthic shoot producing a colorful inflorescence.

Heliconia rostrata Ruíz and Pavón is a musoid plant, with a red and yellow hanging inflorescence, from the Amazonian area of Ecuador, Brazil and Peru (Berry and Kress, 1991). *H. rostrata* has been reported growing under Ecuadorian wild conditions from 200 m to 900 m altitude. This species seems to be primarily a swamp and riverside plant (Andersson, 1985). The climatic conditions at the Equatorial Amazon area is characterized by stable temperatures throughout the year (around 20 to 25 °C at 1000 and 500 meters above sea level, respectively), and high rainfall (above 2400 mm/year) with dry periods occurring from December to February and August (Renner et al., 1990).

The shoots of this medium-sized heliconia species that can reach 3 m high and has distichous leaf arrangement. Leaves are divided into a blade, petiole and sheath. The sheaths are open and overlapping to form the pseudostem or aerial shoot. The floral apex is formed by the transition of previously vegetative apex. An inflorescence terminates

each axis, although some apices abort during inflorescence production. The inflorescence is pendent and consists of a number of red-yellow boat-shaped bracts distichiously arranged, each containing a cincinnus (monochasium) of several flowers that flower acropetally (Kress, 1984).

The blooming behavior of *H. rostrata* under wild conditions has not been reported. However, Berry and Kress (1991) pointed out that *H. rostrata* could bloom all year-round, while Criley and Broschat (1992) reported it as seasonal plant, blooming from March to July in higher latitudes.

Inflorescence initiation in heliconias occurs in the apex without obvious external evidence of this process until inflorescence emergence. However, morphological and anatomical changes at the shoot apex during transition from vegetative to reproductive, in many plant species, indicate that flower formation is usually accompanied, or even preceded, by several changes which are often regarded as "symptoms" of flowering. The most common are: stem elongation, leaf growth, changes in leaf shape, phyllotaxis, rate of leaf primordium initiation, precocious initiation of axillary buds, and changes in meristem size and form (Bernier et al., 1981a).

The onset of the reproductive stage is of great practical and theoretical interest. In order to manipulate the flowering in heliconias, it is essential to study when and how the initiation and development of the inflorescence occurs and what factors affect these processes. The changes occurring at the anatomical and morphological level previous to and during the inflorescence development in *H. rostrata* have not been described. Microscopic observations of the apex, on different dates and plant growing stages previous to the seasonal blooming, were carried out to determine the actual status

(vegetative or flowering phase) of plant development and to describe the morphological changes that occur in the shoot apex during the transition from vegetative to floral. Inflorescence development was also studied, since heliconia shoot apex death was reported to occur in *H. stricta* (Lekawatana, 1995) during floral differentiation.

3.3. Materials and Methods

Clumps of *H. rostrata* 'Five Days Peru' growing in the Manoa area at the Harold Lyon Arboretum, the Horticulture department Magoon research facility, and on Manoa Campus at the University of Hawaii between October, 1996 and March, 1997 were observed. The beginning of inflorescence emergence, defined as the compressed bracts appearance out of pseudostem, was recorded.

The number of expanded or unfurled leaves (one to eight) was used as an external marker or reference of the plant growth stage, and the pseudostem length from ground level to the overlapping of the last leaf sheath was recorded as plant height. Over 160 plant samples at various growth stages were taken from a same clump at the Lyon Arboretum on different dates during two preseason periods (97-98 and 98-99) in order to examine their apices under a light microscope. The plants were harvested at ground level from a clump growing under natural shaded conditions. Dissection of the pseudostem was performed in order to locate the apex above ground level, and to count the number of furled leaves growing inside the pseudostem (outside of the apex area) before removing the apex.

The apices were fixed in FAA (1.8 % formaldehyde, 5 % acetic acid, and 45 % ethanol) and labeled. Following a standard technique (Johansen, 1940), the samples were dehydrated in a graded series of ethyl alcohol-tertiary butyl alcohol solutions, infiltrate,

and embedded in Paraplast®. Longitudinal and transverse sections were made on a rotary microtome from 15 to 8 μ thickness respectively, and stained with 1 % safranin and 1 % fast green FCF (Johansen, 1940). The samples were dehydrated with ethanol transferred to xylene and mounted with a permanent mounting medium (Permount from Fischer Scientific Company). The sections were viewed through a light microscope and photomicrographs were taken.

The vegetative and reproductive status of the apex were determined and the numbers of leaves, primordia, and bracts were counted. The total number of leaves per plant was determined by adding together the expanded or unfurled leaves, the furled leaves growing inside of the sheaths that form the pseudostem (> 1 cm), and the recently formed leaves (< 1 cm other than primordia) covering the apex.

3.4. Results and Discussion

3.4.1. Blooming season

None of the plants in the clumps of *H. rostrata* located at Lyon Arboretum, Horticulture department research facility, or Manoa Campus, showed any external signs of blooming between October 1996, when the observations started, and February 1997. Early inflorescence emergence was observed during the second half of February at the Horticulture research facility and Manoa Campus, and in the first week of March at the Lyon Arboretum. The later emergence at the Lyon Arboretum may be due to the lower light and temperature conditions (not measured).

The blooming behavior of *Heliconia rostrata* supports the pattern reported by Criley and Broschat (1992). Flowering seems to be in response to environmental and

internal signals in this species. Responsiveness to photoperiod can form an effective strategy. It allows synchronization between the life cycle of the plant and seasonally associated changes in the environment (Attridge, 1990).

3.4.2. Shoot characteristics

Plant allometric characteristics with relation to terminal apex development of *H. rostrata* were determined on the samples collected on the different dates at the Lyon Arboretum and referenced to the apex status.

Leaves. The average number of furled leaves larger than 1 cm or growing inside of the pseudostem was one; while the number of leaves (smaller 1 cm, apart from the primordia) covering the apex varied from zero (at the reproductive stage) to three leaves. For example, when the plants have one expanded leaf, the total number of leaves (one leaf inside of the pseudostem plus two leaves covering the apex) was at least four. When the growing leaf began to emerge from the pseudostem, the next leaf started to grow inside of the pseudostem.

An important observation was that plants in January, which were growing under probable inductive conditions, had fewer leaves covering the apex than plants under non-inductive condition. Figure 3.1 shows that a maximum of two leaves covering the apex (> 1cm) was found in plants with one to three expanded leaves, and only one to zero (at the reproductive stage, not shown in Figure 3.1) after five leaves had expanded. On the other hand, the number of leaves covering the apex was consistently two or higher for all the plant stages in plants before December first (10 h 56' daylength). The decreasing number of leaves at the apex was the result of the expansion by January of December's furled leaves, and the apex stopped differentiating more leaves, presumably to change to bract

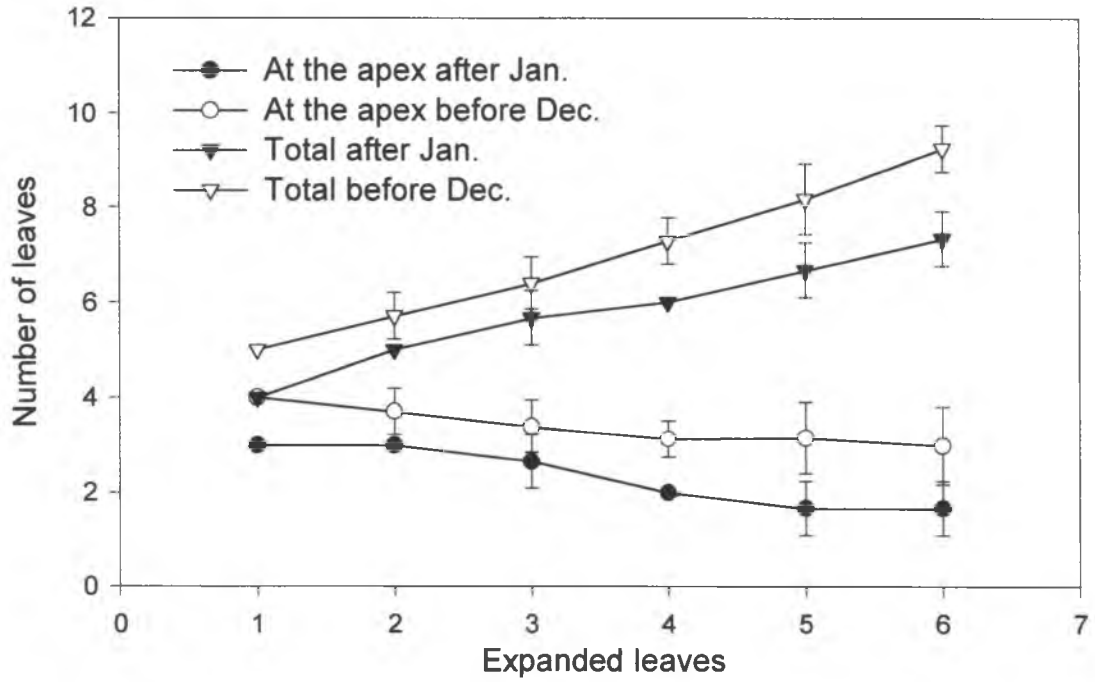


Fig. 3.1. Total of expanded and furled leaves on *H. rostrata* pseudostems in December (prior to reproductive development) and in January (subsequente to reproductive development).Vertical bars are standard deviations.

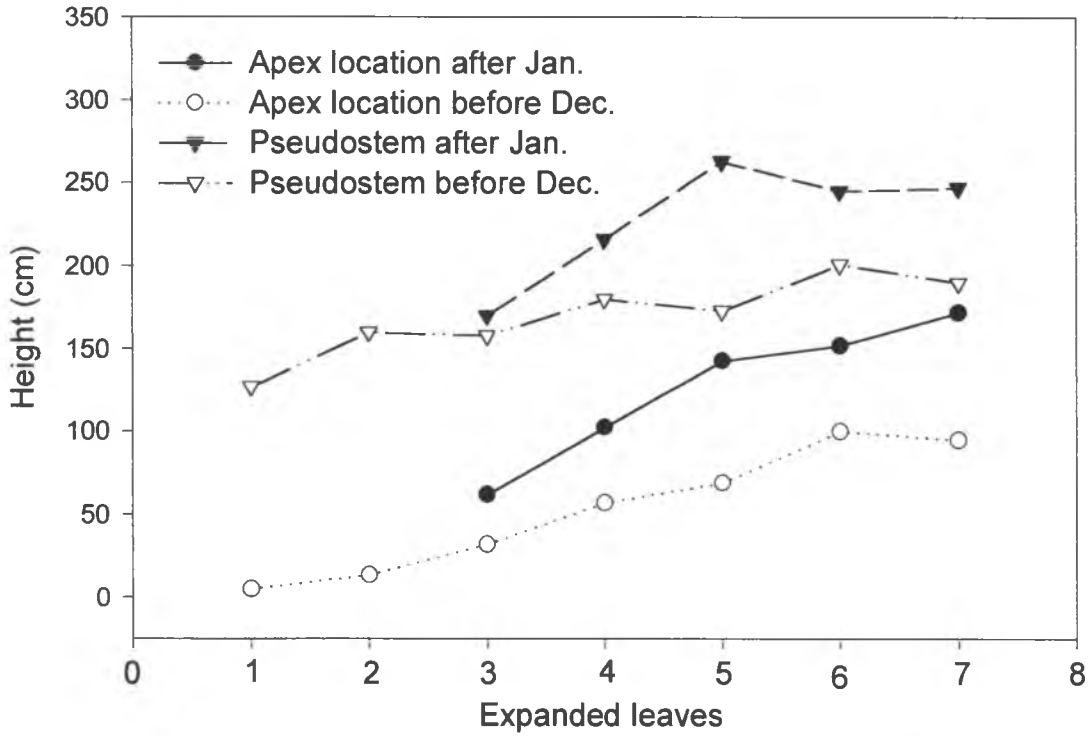


Fig. 3.2. Pseudostem height and apex location of *H. rostrata* in relation to expanded leaf number previous to and after the reproductive stage (before December and after January, respectively).

and flower production. These observations suggest that the number of leaves covering the apex may be an index of synchronization of growth having potential use to study the transition phase of the apex.

Each shoot produces five to ten (occasionally more) laminate (foliage) leaves prior to developing the inflorescence. The number of laminate leaves produced was related to the amount of time between a shoot's emergence and when the apex is induced to flower. Shoots developed previous to inductive conditions had more leaves than shoots that developed under inductive conditions.

There was a reduced leaf between the typical leaves and the inflorescence bracts, and the first bract often ended with a laminate appendage. In addition, the first bract was sterile.

Pseudostem height and apex location. The pseudostem height and the apex location above the ground level were directly related to the expanded leaf number (Figure 3.2) in both vegetative and flowering plant stages in shoots of *H. rostrata* sampled from November 86 to January 87. A synchronized relationship between pseudostem height and apex location or bolting of the pseudostem axis (the stem that will support the inflorescence) was clearly observed. Correlation analysis was performed for both variables. The correlation coefficient (r) for the relationship between the pseudostem height (x) and apex location (y) was 0. When the apex was located above 60 cm height, which occurred at three or more unfurled leaves, the flowering stage could be found, but this stage occurred only in plants that had received sufficient inductive conditions, such as the ones sampled in January. Even when the plants sampled from November to

December had their apices located higher than 100 cm, they were still vegetative. And yet they had 8 weeks of day lengths less than 12 hours.

The rapid elongation of young internodes is one of the early signs of the transition to the reproductive stage. This rapid stem elongation or bolting is most obvious in plants that possess the rosette habit, and it is attributed to changes in rate of cell division in the subapical region (Bernier, 1988). Although stem growth and flower initiation are usually associated processes, they can be separated in many plants. In most of the cases, bolting starts before the visible formation of the reproductive structures. Internode elongation may occur, however, without flower initiation; this is often observed in photoperiodic and cold-requiring species grown in noninductive conditions and also in some plants in response to applications of gibberellic acid that do not cause flowering. (Bernier et al., 1981a,b).

In plants related to heliconia, such as banana, the bolting of the apex above the ground level is used as an index for the beginning of the flowering process (Galán and García, 1984). Once the female floral strata are established, in the self inductive banana inflorescence, the floral stalk begins to enlogate (Lassoudiere, 1978 and 1980). Bolting was also considered as potential indicator in *H. bihai* and *H. latispatha*. In both heliconia species, the flowering stage was observed at early bolting, around 12 cm above the ground level in shoots from the fourth generation which developed under natural inductive conditions (Maciel 1991; Maciel and Rojas, 1994).

Although the apex of *H. rostrata* was located at different levels above the soil in different shoots, it remained without identifiable bracts until late December, hence the method of pseudostem dissection and macroscopic observation of apex bolting used to

determine the vegetative and reproductive stages in banana seems not suitable for *H. rostrata*, since the observations do not support a relationship between bolting and development of a reproductive apex. The difference in results between *H. rostrata* and *H. bihai* and *H. latispatha* (Maciel, 1991; Maciel and Rojas, 1994) may be a consequence of sampling shoots in this study from different ramet or generation which mean shoots grown under different daylength conditions.

3.4.3. Apex status

All the microtome-sectioned plants from November and December (1997) were in vegetative stage (Table 1). The earliest reproductive or flowering stage was found on January 7 (period 98-99) in shoots with 8 or more leaves, while shoots with fewer leaves were still vegetative. One week later, January 15, during the same period all (100 %) shoots with 6 or more leaves already were in reproductive stage; while 50 % of the shoots with 5 leaves were reproductive. However, by this date on the previous year (97-98), one shoot with only 3 expanded leaves was already reproductive. Unfortunately, plants with 4 expanded leaves were not available to sample during 97-98. By January 29 and February 14, period 98-99, 100 % of the shoots with 5 or more leaves were reproductive. Also, 50 % of shoots with 4 leaves were reproductive.

Since the analysis of 1997-1998 year was done using long intervals of time between sampling dates, the beginning of the flowering stage was not conclusive. The results of 1998-1999 observations, besides confirming the plant status of the previous year, allowed improved accuracy in the dating of the reproductive stage. The comparison of 5- and 6-leaf plants versus those with 4 leaves also showed that a plant with greater

Table 3.1. Microscopic apex status determination in *H. rostrata* growth under natural inductive conditions over the periods 1997 to 1999 at Manoa.

Period	Sampling date	Number of samples	Leaf count range	Apex Status[1]	Comments
97-98	Nov. 01	13	1 to 8	Veg.	100% of samples
	Nov. 15	12	1 to 6	Veg.	100% of samples
	Dec. 01	10	2 to 7	Veg.	100% of samples
	Jan. 15	12	1 to 8	Rep.	Some shoots >3 leaves
98-99	Dec. 22	12	2 to 8	Veg.	100% of samples
	Jan. 07	12	3 to 9	Rep.	Some shoots ≥ 8 leaves
	Jan. 15	47	1 to 9	Rep.	50% of shoots with 5 leaves; 100% of shoots ≥6 leaves
	Jan. 29	20	2 to 7	Rep.	50% of shoots with 4 leaves; 100% of shoots ≥5 leaves
	Feb. 14	22	2 to 8	Rep.	50% of shoots with 4 leaves; 100% of shoots ≥5 leaves

[1] Veg.= Vegetative; Rep.= Reproductive

number of leaves reaches the reproductive stage earlier than the ones with lower number. It suggests a higher susceptibility to be induced for plants with greater numbers of leaves. Similar behavior has been reported for other plant species (Thomas and Vince-Prue, 1997). In addition, the equal percentages of flowering and plant stage from plants in January 29 and February 14, which represent 2 weeks difference of growth, suggested that 50% of floral apices on shoots with 4 expanded leaves may be the maximal percentage to be reached at this stage of growth after enough inductive conditions or under continuous inductive conditions.

Figure 3.3 summarizes the different stages of the apex, the location of the apex (as a percentage of pseudostem height), and the minimal number of bracts for each stage at two dates of sampling for the period 98-99. The transition (yellow arrows in Figure 3.3) from vegetative to reproductive was reached in elongated pseudostems (apex located >10% of the pseudostem height) in both dates. Shoots with three leaves were already in transition in February; while that transition was delayed in January samples until the shoot reached four leaves. The onset of the reproductive stage (first reproductive bract) occurred one leaf later (i.e. more expanded leaves were necessary in January than in February to reach a transitional stage). The inflorescence is hidden throughout most of its development. From 8 to 12 weeks are estimated for its development from the first bract to emergence from the pseudostem, since, the earlier inflorescence emerged at the beginning of March, and the earlier reproductive apex was observed on samples from early January.

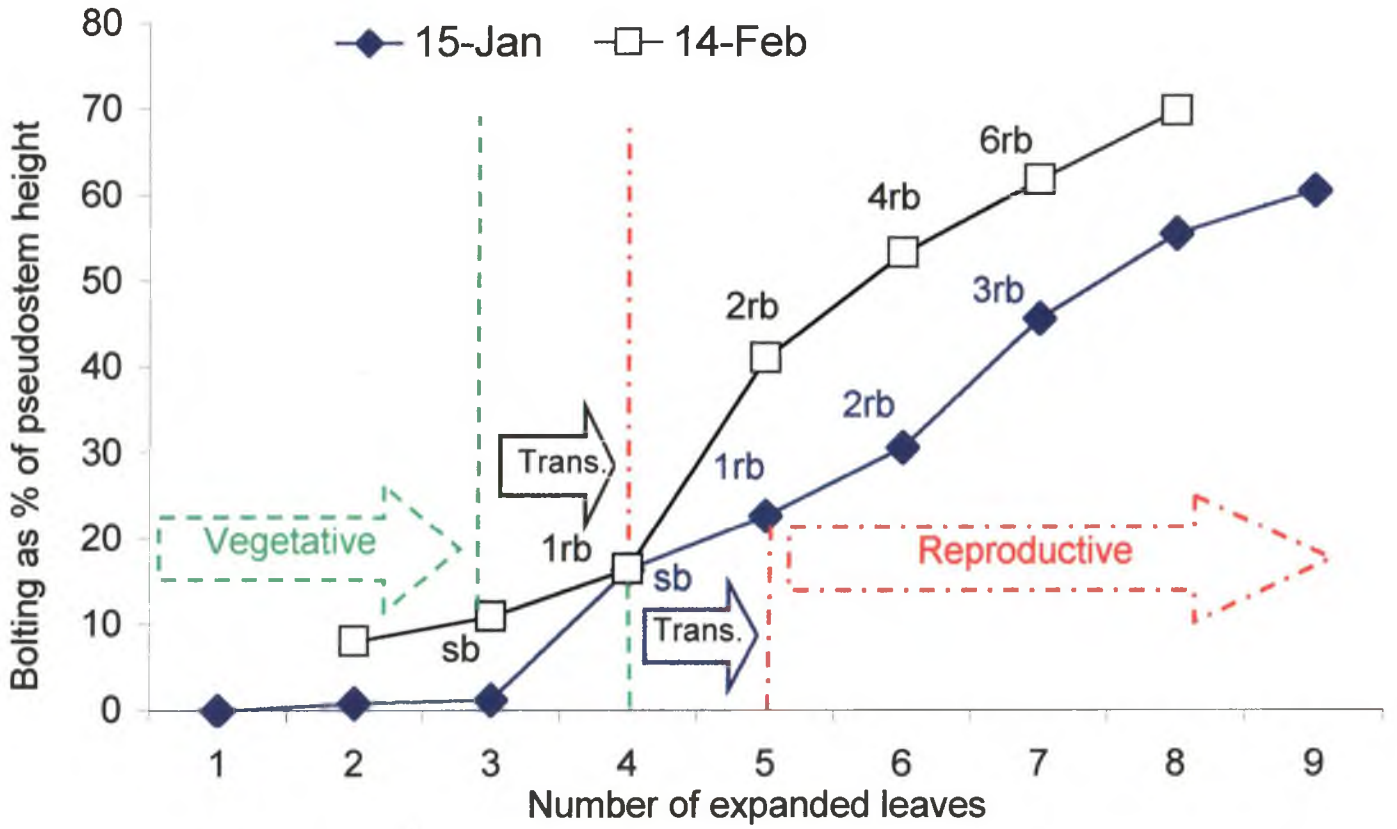


Fig. 3.3. Status of apex, number of bracts and bolting (apex location) as a percentage of pseudostem height in *H. rostrata* at two dates. N = minimum of 3 samples per point. Sb, sterile bract; rb, reproductive bract; trans., transition.

Figure 3.3 also shows that with the increase of the number of expanded leaves there is an increased minimum number of bracts. This tendency is independent of the date of sampling. Nevertheless, shoots with the same expanded leaf number have more bracts in the sampling of February 14 than in January 15. This may be a consequence of earlier induction and/or growth for longer periods under inductive conditions.

Inflorescence initiation occurs in *H. stricta* and *H. angusta* when plants have a minimum of 3 or more leaves under inductive conditions. The plant remains vegetative and continues to produce leaves (up 8 to 9) under non-inductive conditions (Criley and Kawabata, 1986; Kwon, 1992; Lekawatana, 1986). Because some of *H. rostrata* plants collected before December already had enough leaves to support flowering, it was hypothesized that even though plants have the potential to flower, they do not do so; instead leaf differentiation continues until the necessary stimuli or threshold for inflorescence initiation and development are reached. These results demonstrate that some environmental factors must be present in order for the apex to change from vegetative to reproductive stage. Also, some intrinsic plant characteristics are required. For example, the number of leaves per pseudostem has been reported to decline with the ensuing generations in species such as *H. bihai* and *H. latispatha* (Maciel, 1991; Maciel and Rojas, 1994)); this may be obvious for seasonal species growing under non-inductive conditions, but it also occurs in the aseasonal *H.* 'Golden Torch' cultivated in pots (Catley and Brooking, 1996a,b).

Some heliconia species appeared to require a minimum leaf number in order to be induced. If the minimum number of leaves in a flowering shoot of *H. rostrata* is five,

such as it has been found in plants flowering (reaching anthesis) at the end of the season (data not presented), one plant with three unfurled leaves and synchronized growth already has five leaves (one inside of the pseudostem <1 cm and one covering the apex). Thus three unfurled leaves is the minimum number necessary to switch from vegetative to reproductive stage in *H. rostrata*. Although leaf number is not the only condition required.

3.4.4. Anatomy

Vegetative apex. A vegetative apex is domed (Fig. 3.4). The dome varies from aproximately 100μm to 160 μm in height and from 180μm to - 340μm in width. The main evidence of activity is just within the flank of the meristem where the primordium for the next leaf appears. Each new leaf appearance alternates to the right and to the left of the central meristem forming a distichous pattern. One leaf originates on the apex at any time, and when the next primordium is initiated, the meristem is surrounded by the base of the most recently formed leaf primordium. Although *H. rostrata* has one axillary vegetative bud, this is only obvious far from the tip of the shoot where the pseudostem joins the rhizome.

The arrangement of leaf bases can be visualized as a series of cones, each fitting precisely over the next one to be formed. In a median longitudinal section, perpendicular to the leaf axils, similar portions of the same leaf appear on the two sides. In sections parall to the leaf axis the base may be observed only on one side, where the vegetative axillary meristem bud will be observed.

During vegetative growth there are few primordia present at any given time at the apex. The apex initiates the leaf primordia, and these subsequently grow by the activity of

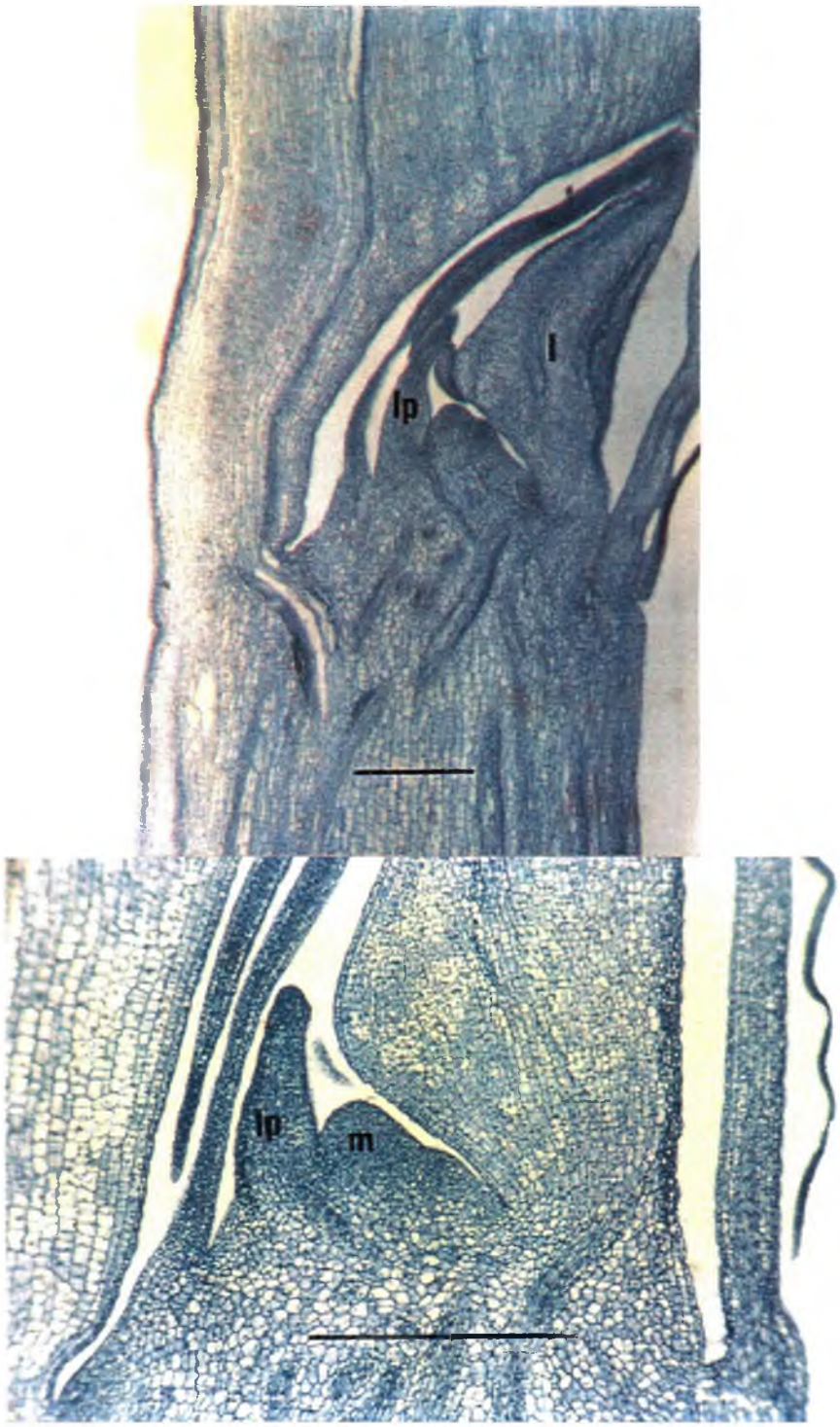


Fig. 3.4. Median longitudinal section of the vegetative apex of *H. rostrata*. Bar equal 400 μm. l=leaf, p= primordium, m=meristem

special meristematic regions and scattered cell divisions. Thus, the structure of the aerial shoot in *H. rostrata* is built, not by the apical meristem itself, but mainly by its appendages, as in the banana plant (Baker and Steward, 1962). This structure is called pseudostem.

As in most monocotyledons, the vegetative buds of heliconia are lateral and occur in the axil of the foliar structure (Fisher, 1978). The earliest evidence of a bud is a deeply stained region in the axil of the second or third primordium. However, a shell zone may or may not be present until the fourth primodium (Fisher, 1978).

Transition. With the transition to inflorescence, the apical region elongates and more phyllome primordia are observed at the apex (Fig. 3.5). At this stage, bracts occur, but no axillary bud primordia are visible. Unlike a foliage leaf, the bract does not surround the apex previous to the initiation of the next primordium, nor does it occupy its circumference when observed in a cross-section at the apex.

The transitional stage, where bracts occur but no flower primordia, conveys a different impression than the vegetative apex. The increased number of leaf primordia or phyllome was associated in banana with a decrease in the insertion size of phyllome (Baker and Steward, 1962). Similaraly in heliconia, the elongation from vegetative to the floral shoot is accomplished long before visible floral parts are present, or even before the axillary buds which form the flowers have appeared.

Inflorescence

The inflorescence apex is shallowly domed and produces bracts on the side of the dome (Fig. 3.6). The free apical dome is tilted in the direction in which the next bract will

Fig.3.5. Median longitudinal section of shoot apex of *H. rostrata* during transition from vegetative to floral. Bar equal 400 μm. l=leaf; lp=leaf primordium; m=meristem.

Fig.3.6. Median longitudinal section of early floral apex of *H. rostrata*. Bar equal 400 μm. rb=reproductive bract; sb=sterile bract; bp=bract primordium; cp=cincinnus primordium.

appear. A floral bud formed in the axil of the bract is easily observed at the third bract primordium below from the meristem.

The bracts appeared in rapid succession with active growing regions in every bract axil (Fig. 3.7). Although both leaf and bract primordia are arranged distichously on their respective axis the bracts are much more crowded than the leaves. Thus, the inflorescence apex appears larger than the vegetative apex because the youngest bracts do not enlarge as early or as rapidly as the vegetative leaves. The axillary buds form precociously and closer to the apex in reproductive apices as compared with vegetatives ones. This has been associated, in banana, with loss of apical dominance (Baker and Steward, 1962; Mohan Ram et al, 1962).

After initiation, each axillary bract bud primordium enlarges to produce a cincinnus (Fig. 3.7). Flower development begins later with the transformation of the cincinnus apex into a floral primordium. Since, the floral bud is initiated as floral apex in the axils, it never passes through a vegetative phase.

The heliconia inflorescence growth and maturation is acropetal. The inflorescence terminates through the cessation of growth of the apex. As the inflorescence ages, the inflorescence apex decreases in size relative to the surrounding bracts and finally ceases growth.

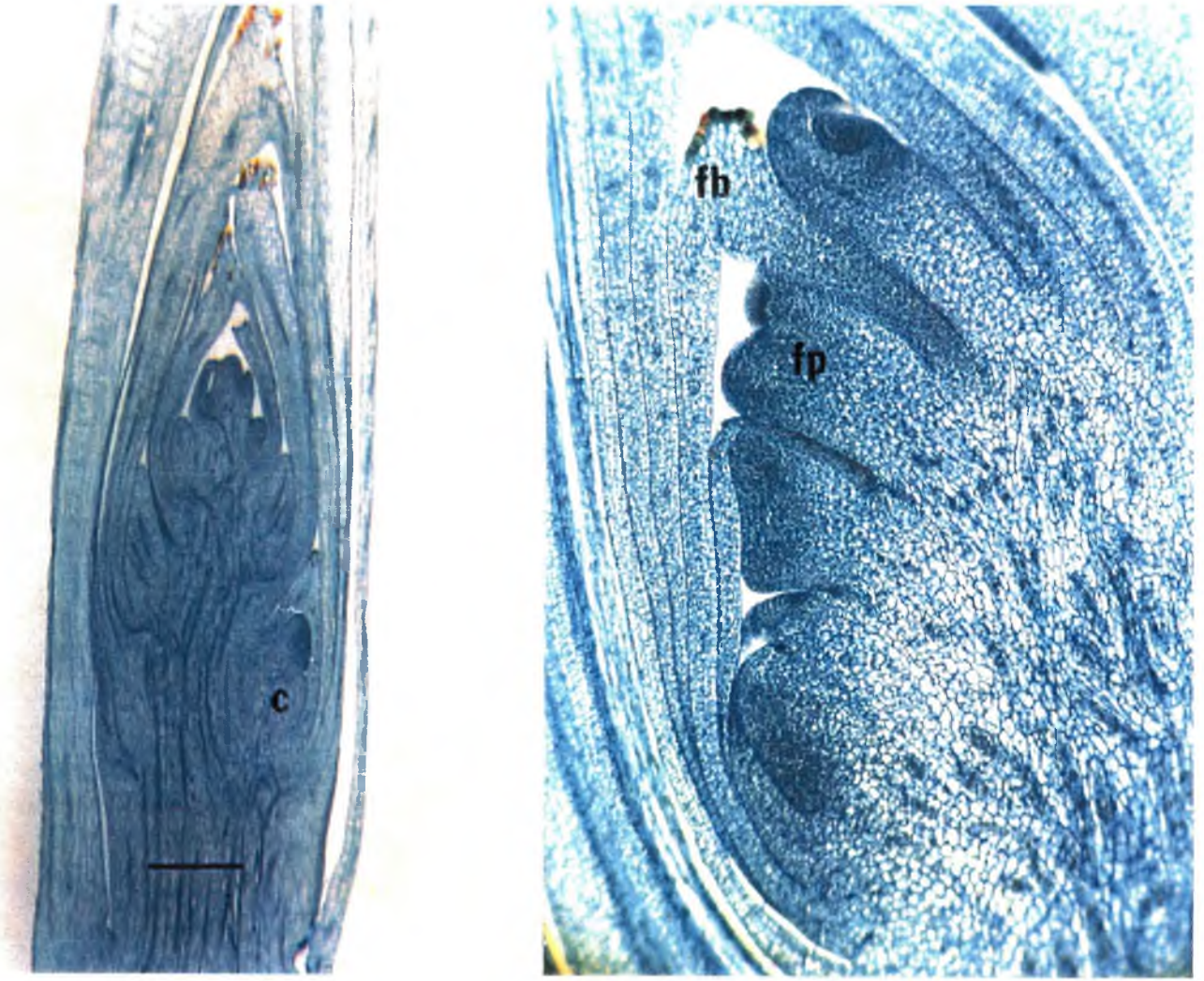


Fig.3.7. Median longitudinal section of floral apex of *H. rostrata*. Bar equal 400 μm.
c=cincinnus; fb=flower bract; fp=flower bract.

3.5. Conclusions

The seasonal blooming of *H. rostrata* begins in March in Hawaii and occurs at least 12 weeks after the most incipient inflorescence was observed microscopically. The short days previous to the determination of the floral stage suggest photoperiod as the environmental trigger of flower induction.

Each shoot bears a variable number of leaves subtending the inflorescence. The number depends on the time between shoot emergence and exposure to the flowering stimulus. The earliest inflorescence was observed in shoots with at least three unfurled leaves that had received sufficient inductive stimulus.

Expanded leaf number, pseudostem height and the apex location above the ground level were in direct and synchronized relationship in both plant stages (vegetative and floral). Therefore, the dissection of the pseudostem to determine if the shoot is floral only by looking to the apex location need to be carefully used. This method might be applicable in shoots that had developed under inductive conditions, but not when the plants were under not inductive factors.

The more obvious morphological changes during the transition of *H. rostrata* are: change in primordium form (bract formation), the rapid succession of bract primordia appearance (this is in marked contrast to the fewer leaf primordia observed in the section close to the tip), and the precocious axillary bud growth closer to the apex (which gives origin to flowers).

The reproductive status was easily detected under the microscope when the inflorescence had at least three bracts. Flower differentiation on the cincinnus begins when many bracts are well developed.

Since the time period from differentiation of the first floral primordia to anthesis takes more than 12 weeks and does not consider the time frame for induction and transition of the apex. Then, the overall process from induction to anthesis must be longer in this species than in *H. stricta* which require 12 weeks.

CHAPTER 4

DAYLENGTH AND NIGHT TEMPERATURE ON THE INDUCTION OF FLOWERING AND GROWTH OF *HELICONIA ROSTRATA* RUÍZ & PAVÓN

4.1 Abstract

Plants of *Heliconia rostrata* were subjected to five different daylength treatments (9, 9.5, 10, 10.5, and natural daylength >13 hours) to analyze the effect of photoperiod in the induction of flowering, in a first controlled experiment. The treatments were applied by moving the potted plants in the evening from the glasshouse to a dark chamber at 23 °C every day for eight weeks. Plants subjected to photoperiods of less than 10.5 hours flowered, whereas the ones growing under natural daylengths (>13 hours) did not. The emergence of the inflorescence occurred between 21 and 28 weeks after the onset of short days. Only shoots with three or more unfurled leaves at the end of SD treatment flowered. The results support the hypothesis that *H. rostrata* is induced by short days.

In a second experiment, plants were subjected to all combinations of three daylength treatments (9.5, 10.5, or 11.5 hours) during 4, 5, 6, 7, or 8 weeks in order to determine the interaction between daylength and number of weeks that induce flowering. The critical daylength was not determined under this experimental condition, however, it was greater than 11.5 hours. Flowering occurred in all the treatments. Even though no significant differences were detected, more flower shoots were observed at longer daylengths for of 6 or more weeks.

In order to determine the effect of night temperature and its interaction with daylength in flowering of *Heliconia rostrata*, plants were subjected to a factorial experiment with both factors at three levels in a third experiment. The daylengths used were 9, 11 and 13 hours, and the temperatures were 16, 21 and 26 °C. Flowering occurred at 9 and 11 hours, but did not occur in plants subjected to 13 hours daylength. This demonstrated that night temperature per se did not induce flowering in this species. Even though no statistical differences were detected among temperatures, fewer shoots flowered (12.5 %) at 26 °C than at 16 and 21 °C (37.5 and 50 % respectively). In addition, there were differences for the total of shoots among and between daylength and temperature, which were mainly related to vegetative development after the inductive treatments.

In conclusion, the flowering of *H. rostrata* can be manipulated with short days. Shoots with at least three leaves were induced to flower when subjected to photoperiods less than or equal to 11.5 hours for periods of four or more weeks. Increasing the number of weeks under short days improved the number of floral shoots. Night temperature in the range of 16 to 26 °C had no effect in inducing flowering. However, flowering shoots number decreased as night temperature increased from 21 to 26 °C during the induction. In all experiments the dead of shoots affected inflorescence production of this species.

4.2 Introduction

The value of critical daylength (CDL) that marks the transition between vegetative growth to flowering in obligate photoperiodic plants of both SD and LD types varies considerably between species and cultivars, environmental conditions and plant age and/or size (Thomas and Vince-Prue, 1997). Critical photoperiods close to 12 hr have

been observed in certain varieties of sugar cane and rice. When seasonal flowering plants evolved at low latitudes, they were able to perceive small changes in the daylength (Zeevaart, 1976). On the other hand, the range of photoperiods over which plants change from vegetative growth to maximum flowering can also vary with the number of inductive cycles given (Thomas and Vince-Prue, 1997).

The critical daylength and the number of inductive cycles are important to program flowering in photoperiodic species of heliconia. Among the photoperiodically studied species, *H. stricta* 'Dwarf Jamaican' is a SD small plant that is induced to flower under inductive daylengths of 8 or 9 hours given for at least 4 weeks (Criley and Kawabata, 1986; Lekawatana, 1986). In *H. angusta*, a LD plant, at least 7 weeks with a minimum of 13 hours of daylength was required for flower induction. However, more plants flowered at 8 and 9 weeks of treatment than at 7 weeks. Since no flowering occurred in plants of *H. angusta* growing below 12.5 hours daylength, it was hypothesized that this species has a critical photoperiod requirement between 12.5 and 13 hours (Kwon, 1992).

Temperature changes are known to interact with photoperiodic processes by shifting phase and amplitude, thus initiating rhythmic responses in plants (Searly, 1965). Even small changes in temperature may suffice to alter the plant response (Bernier, 1988). There is evidence that decreasing temperatures progressively nullify the daylength requirement of absolute LD or SD plants (Bernier et al., 1981 a). However, the opposite effect has also been reported (Kinet, 1993). Early anthesis of pearl millet has been induced through manipulation of photoperiod and temperature (Hellmers and Burton, 1972). Besides photoperiod, temperature is another factor that may play a role in

induction of heliconia. Lekawatana (1986) reported that, with increasing night temperature during the induction period, there were fewer shoots that flowered in *H. stricta* 'Dwarf Jamaican'. The percentage of flowering shoots decreased from 53% to 14 % as night temperature increased from 15 °C to 25 °C during the 4 weeks of SD; while the percentage of vegetative shoots increased from 16% to 48%. The effect of the interaction between daylength and temperature was not reported.

A minimal number of leaves must be unfurled on the pseudostem of heliconia before the apex is capable of responding to a inductive stimulus. In *H. stricta* and *H. angusta* the minimum number of unfurled leaves required for the shoot to be induced was three leaves (Kwon, 1992; Lekawatana, 1986; Lekawatana and Criley, 1989). In *H. latispatha*, five leaves were unfurled when the reproductive apex was early-determined (Maciel, 1991; Maciel and Rojas, 1994). Besides species variability, the minimal number of leaves also varies with the environmental conditions and the successional occurrence of the shoots in the clump, at least for the first generations after planting. In shoots from a fourth generation of *H. bihai* that were growing under natural inductive daylength and two shade conditions (60 % and 0 %), the reproductive stage was observed microscopically when a minimum of four and five leaves were expanded, respectively (Maciel, 1991; Maciel and Rojas, 1994).

The studies in plants of *H. rostrata* growing under natural daylength (Lyon Arboretum) presented in the previous chapter suggested that its seasonal blooming is photoperiod related. Since the transition and subsequent blooming occurs after the natural short days, it was hypothesized that *H. rostrata* is a short day plant. Controlled studies of the flowering behavior of *H. rostrata* have not been reported, hence three experiments

were designed to: Analyze how different photoperiods affect flowering; determine the critical daylength and minimum number of inductive photoperiod cycles required; and determine how nocturnal temperature affect induction. The results of these experiments could be used to schedule artificial induction of flowering in *H. rostrata.*

4.3. Materials and Methods

These experiments were conducted at the facilities of the University of Hawaii at Manoa.

4.3.1. Effect of daylength (Exp. 1.)

Rhizomes were potted into plastic containers (18 cm diameter x 15 cm depth) containing a mixture of peat, perlite, composted red wood, and volcanic cinders at 1:1:1:1 proportions, and amended with dolomite, Micromax^{TM} (minor elements) and treble superphosphate at ratios of 6.0, 1.0 and 0.6 kg/m^3 respectively during Summer 1996. The plants were grown at Magoon Horticulture research facility, under full sunlight and with extended daylength (6:00 to 10:00 p.m.) provided by incandescent 60 w lamps placed 1.6 m above the bench (1.9 Wm^{-2}) from October to February. Plants were automatically drip-irrigated with nutrient solution (200 N- 0 P- 233 K ppm) at a rate of 1000 ml per pot per day.

In summer 1997, the plants were moved into a glasshouse at Pope laboratory, and only shoots with quantifiable number of leaves were left in the pots. Their unfurled leaves were counted and tagged at the beginning of the short-day treatments on July 1. During this experimental phase, the plants were watered daily by hand, and fertigated twice a week with 1000 ml/pot of nutrient solution of 500 ppm N-P_2O5-K_2O at the ratio of 20-20-20.

Four short daylength treatments, 9, 9.5, 10 and 10.5 hours, were provided by moving the plants on carts at different times (4:30, 5:00, 5:30 and 6:00 p.m.) into a 22°C dark chamber for 8 weeks, from July 1 to August 26. Plants were moved back to the glasshouse at 7:30 am. The control plants received natural daylengths ranging from 13h 25' to 12h 52'. A total of fifteen pots, three for each treatment, were used. The average daytime air temperature in the glasshouse was 34 °C, while the average night air temperatures was 21°C after the onset of inductive period. New unfurled leaves were tagged every two weeks until the end of the experiment, and the inflorescence emergence defined as the exposition of the first bracts was recorded each week.

The experiment was concluded on January 30 1998, and the number of leaves per pseudostem was determined. The status of the labeled shoots was determined as flowering, vegetative, or dead. The dead stage was determined by dissecting the pseudostems for which leaf emergence had stopped. In these pseudostems the apical region typically was blackened.

4.3.2. Effect of daylength and number of weeks (Exp. 2.)

H. rostrata rhizomes were potted, on May 1998, into pots (18 cm diameter x 15 cm depth) with the same mixture and amendments described in the previous experiment. The plants were grown in a saranhouse with 20 % of shade at Magoon Horticulture research facility until they reached 3 to 4 leaves. On July 15, the plants were moved to Pope laboratory glasshouse where the daylength treatments were initiated.

Short day treatments were supplied by moving the plants from the glasshouse to a dark chamber (21°C), at different times of the day and for a variable number of weeks. Three different daylengths (9.5, 10.5 and 11.5 hours) and five different periods of SD

duration (4, 5, 6, 7, and 8 weeks) (3 x 5 = 15 treatments) were applied. To accomplish daylength treatments, the plants were moved into the dark chamber every day at 5, 6 and 7 pm, and moved back to the glasshouse at 7:30 am the next day. On September 16, one week after the end of daylength treatments, the plants were relocated to a Magoon glasshouse where extended daylength (>13 hours) with 60 w incandescent lamps separated 1 m apart and placed 1.6 m above the bench (1.9 Wm^{-2}) was supplied to all the treatments from 5 pm to 8 pm until December 1.

During the SD inductive phase, the plants were sprinkler irrigated twice a day and fertirrigated twice a week with 1000 ml/pot of nutrient solution of 500 ppm N-P_2O5-K_2O at the ratio of 20-20-20. The average day air temperature was 36 °C, while the average night air temperatures was 22°C. After the inductive period, the plants were drip-irrigated automatically with nutrient solution (200 N-0 P-233 K ppm) at rate of 1000 ml pot per day. The average day air temperature was 33 °C, while the average night air temperatures was 20°C.

Each treatment consisted of 6 pots (for a total of 90). Each pot per combination of daylength x duration was considered a replicate. Fourteen additional pots were used in the experiment. Six pots were grown under natural daylength conditions to observe when natural flowering occurred. Eight pots were subjected to the shortest daylength (9.5 hours) and the longest inductive period (8 weeks) to analyze the shoot apex by sampling two pots for microscopic examination at weeks 6, 8, 10 and 12 after the onset of SD.

The number of leaves per plant at the onset of the daylength treatments were counted and each new leaf recorded weekly during the first 8 weeks from the onset of the inductive treatment and every two weeks after that until the end of the experiment. At the

end of the experiment, the status of the shoots (flowering, growing and dead) was determined. Pseudostem dissection was used to observe the apex in vegetative and dead shoots. The time to flowering since the application of the treatments and the minimum number of leaves required for the shoot to be sensitive to induction by daylength were determined. The time when the leaf emergence stopped was also determined for dead shoots.

4.3.3. Effect of temperature and daylength (Exp. 3.)

Rhizomes of *H. rostrata* were potted in August 1998, into plastic containers (20 cm diameter x 16 cm depth) containing the same potting medium and amendments described in the previous experiments (see 4.3.1).

Plants were grown under extended daylength, from 5:00 to 7:30 p.m., provided by glasshouse ceiling flood incandescent reflectors (19 Wm^{-2}) at Pope facilities from September 22 to December 1. From December 1 to January 27 the extended daylength at the glasshouse was supplied by the same source of light from 5:00 p.m. to 9:00 p.m. Plants were irrigated three times a day by spray-stakes. One teaspoon of $N\text{-}P_2O_5\text{-}K_20$ at ratio of 19-6-12 from a slow release fertilizer (Osmocote®) was applied to each pot, and additionally fertigated, once a week with 1000 ml/pot of 500 ppm $N\text{-}P_2O_5\text{-}K_20$ at ratio of 20-20-20. The average day time air temperature was 33 °C (ranging from 26 to 36 °C) from September to January, while the average night air temperature was 20 °C (ranging from 19 to 22 °C).

Beginning December 1, night temperature and daylength treatments were imposed by moving the plants from the glasshouse to three separate dark chambers at 16, 21 and 26 °C, at three different times of the day (5, 7 and 9 pm, and removed at 8 am the next

day) for a period of 8 weeks. The daylength treatments were 9, 11 and 13 hours, respectively. A total of 9 (3 x 3) treatments, each one with 8 pots, were used. Each pot per combination of daylength x temperature was considered as a replicate.

After eight weeks of daylength and temperature treatments, plants were moved to the Magoon facility glasshouse. Because natural daylength from January 28 to April 1 ranged between 11h29' and 12h22', extended daylength was supplied with incandescent lamps from 5 pm to 9 pm in the glasshouse. The average day air temperature was 37 °C (ranging from 30 to 42 °C) from February to July, while the average night air temperature was 22 °C (ranging from 19 to 24 °C).

The number of leaves per shoot was recorded at the onset of the daylength treatments. New unfurled leaves were tagged every week during the treatment period and at two weeks intervals after that until the end of the experiment in July 1999. The status of the shoots (floral, vegetative and aborted) was determined after the blooming period. Dead and vegetative stages were determined by dissecting the pseudostems.

4.3.4 Data analysis

Data collected from all experiments were analyzed for variance, and mean separations were performed by Bonferroni multiple comparisons. Correlation and regression analyses of plant parameters against treatments and between variables were performed whenever appropriate. The statistical process was accomplished using the computer program SYSTAT 7.0® for windows (SYSTAT, 1997).

4.4. Results and Discussion

4.4.1. Effect of daylength (Exp. 1.)

Plants grown under natural daylengths from July to August (13h 25' to 12h 52') did not flower in this experiment, while plants given 8 weeks of SD (daylengths shorter than 10.5 hours which is equivalent to December-January daylengths in Hawaii) did (Figure 4.1). If daylength was longer than 10 hours, the percentage of pots that flowered was lower (33%) than the ones in which SD was 10 or fewer hours (66%). Under 9, 9.5, and 10 hours daylengths 2 out of 3 plants flowered, while at 10.5 hours only 1 pot flowered.

The results seemed obvious with respect to a SD exposure as a factor inducing flowering in *H. rostrata*. However, the ANOVA did not detect differences. This might be consequence of the number of replicates used in the experiment and/or the similar variance among the treatments. This species behaves as short-day species like *H. stricta* and *H. wagneriana* (Criley and Kawabata, 1986; Criley and Sakai, 1997). The critical daylength remains to be determined.

No differences attributable to daylength were found either for the number of leaves or for the pseudostem height (at beginning and end of SD, bolting and flowering) in flowering shoots. The minimum and maximum number of leaves per shoot, labeled at the onset of SD, was 2 and 7 leaves, respectively; while at the end it was 5 and 10 leaves. During the 8 weeks of SD the mean number of leaves per shoot increased from 3.6 ± 1.6 to 6.8 ± 1.3 at the end of SD (Figure 4.2). The rate of leaf appearance, for all the treatments, was constant and fast (0.4 leaf a week) during this 8- week period. The rate of leaf appearance after this period declined. Approximately 12 weeks were required for the

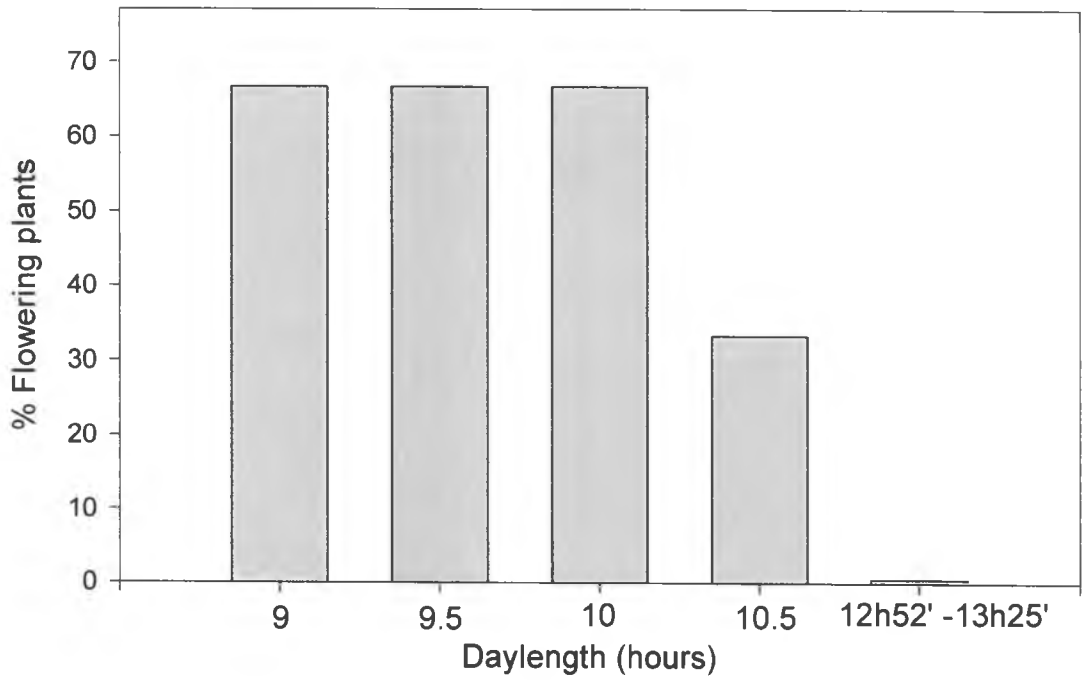


Fig. 4.1. Effect of different daylengths on the flowering of potted *H. rostrata*

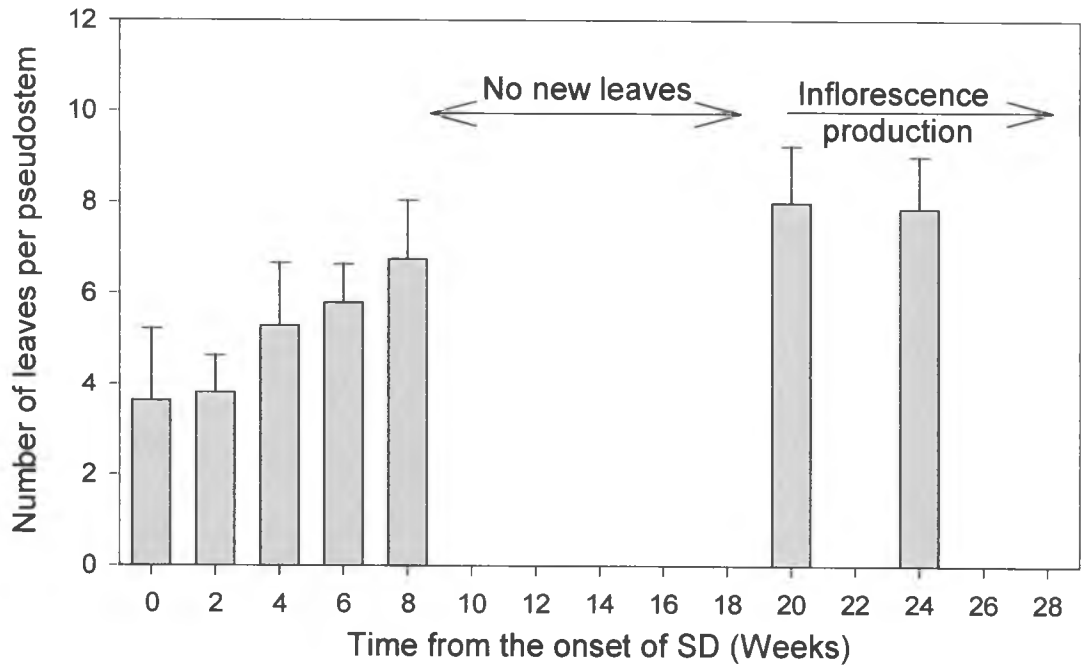


Fig. 4.2. Average expanded leaf count per pseudostem during 28 weeks after the onset of short days. SD was provide for the first 8 weeks. Vertical bars are standard deviation.

next leaf appearance after the 8 week SD exposure ended. The number of leaves subtending the inflorescence (Figure 4.2) ranged from 7 to 9 leaves (mean 8.1 ± 0.9).

The inflorescences emerged between 1 and 4 weeks after the last leaf unfurled, and their occurrence was concentrated in the 6 weeks, from the first week in December through the second week of January, 21 to 28 weeks after the onset of SD (Figure 4.2). The inflorescence emerged earlier (weeks 21 to 23 from the onset of SD) on plants with 4 to 7 rather than with 2 and 3 leaves (24 to 28 weeks after the onset of SD). The number of leaves per shoot at the onset of SD could explain the spread of flowering during 6 weeks. Shoots that had barely produced the minimum number of leaves for induction by the end of the period of induction could flower 6 weeks later than the shoots with more leaves that were, presumably, induced earlier.

If a period of 4 weeks under SD is considered adequate to induce flowering, such as in *H. stricta* (Criley and Kawabata, 1986), and 21 weeks is the minimal period until inflorescence emergence, 17 weeks (approximately 4 months) might be required for inflorescence development.

Previous results from plants under natural daylength (Lyon Arboretum, Chap. 3) suggested that the plant requires a minimum of three leaves to be competent for induction, however, plants with 2 leaves at beginning of SD did flower. This paradox can be explained by the number of weeks under the SD treatments. It was observed that plants with two leaves at the start of SD had developed five leaves by the end of the SD (the minimum number found in flowering stalks). A shorter period of SD could be enough to induce plants with three or more leaves.

The percent distribution of shoots by status (flowered, dead, or vegetative) at the end of the experiment for the 62 pseudostems (100%) grouped by number of leaves at the start of SD is shown in Figure 4.3. Flowering occurred mainly in shoots with 2, 4 and 3 leaves at the beginning of the treatment (25, 25 and 15 %, respectively). No flowering occurred in shoots with 5 or 6 leaves (0%). However, flowering occurred when there were 7 leaves present (11%). Plants with 7 leaves at the onset of SD apparently only developed the leaves previously formed (3 leaves), while plants with 4, 3 and 2 leaves put out 4 or 5. The lower number of expanding leaves in older shoots may be explained by the number of incipient leaves and leaf primordia already formed at the apex and the growth rate of the leaves.

Apex death occurred in shoots with different number of leaves (Figure 4.3). The distribution of dead shoots was 31, 33, 60, 60 and 67% for plants with 3, 4, 5, 6, and 7 leaves respectively. This distribution of dead shoots differed from that reported for *H. stricta* when the highest pseudostem death frequency occurred in plants with one to three leaves (Criley and Kawabata, 1986). Figure 4.3 also shows an inverse relationship between the vegetative and dead shoots, where fewer vegetative shoots correspond with more dead shoots.

The leaf number of shoots at the start of induction seems to be important in determining which shoots will flower and which will die, since shoots with 2 to 4 leaves were the ones that flowered, while most of the ones with 5 to 7 leaves died. Even though the death of apex has been reported as an important factor affecting flower production in heliconias, it is unknown when and what factors induce it (Criley and Kawabata, 1986; Lekawatana 1986 and 1995). In addition, there were also shoots that remained vegetative

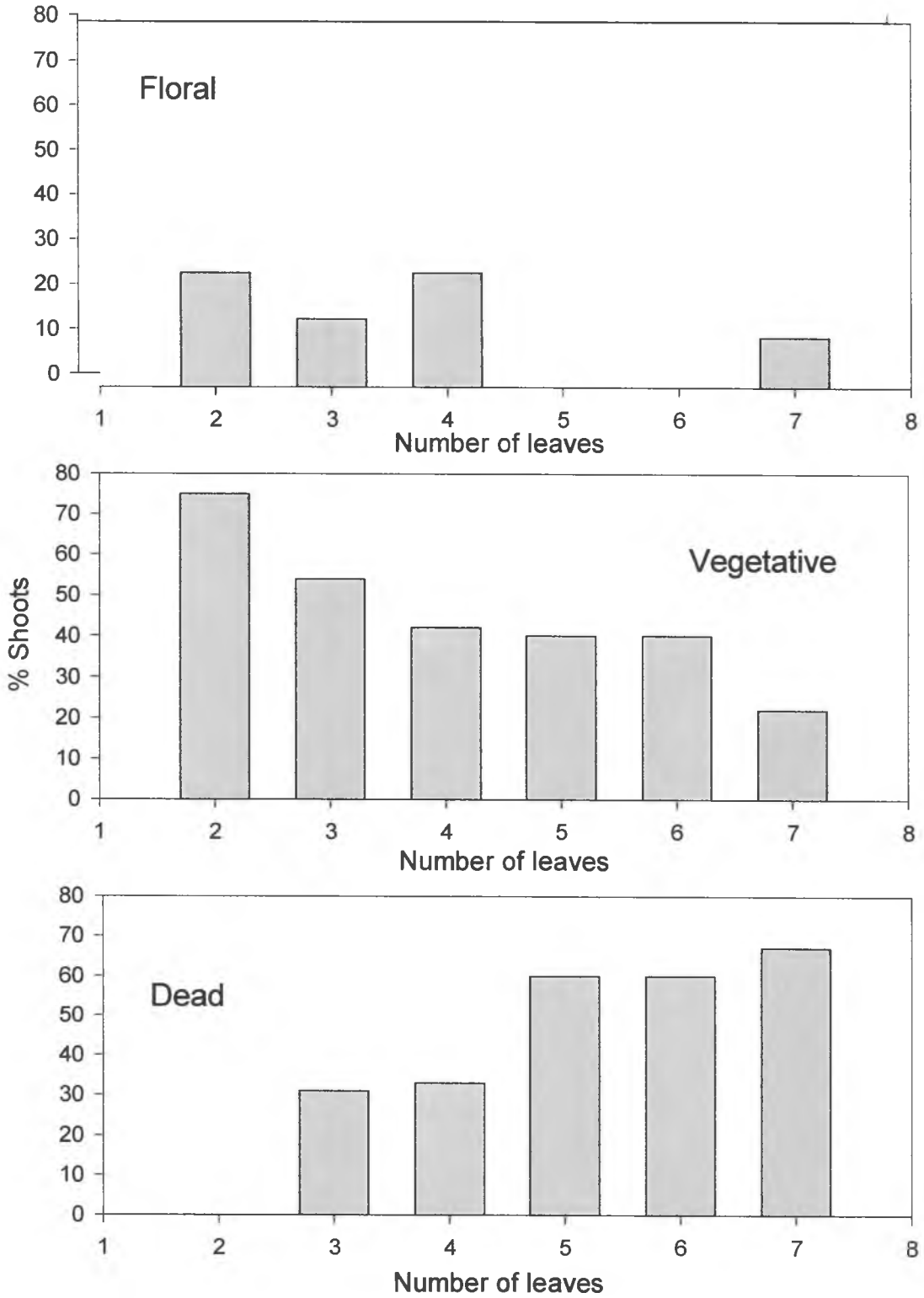


Fig. 4.3. Percent distribution of floral, vegetative, and dead shoots by leaf number at the onset of short days

after the inductive treatment, although they had enough leaves (4, 5, 6 or 7) at the onset of SD to become induced.

The classification of the shoots as flowered, dead, or vegetative at the different daylengths is shown in Figure 4.4. The number of flowering shoots per pot was not high; except for one pot with two flowering shoots, only one shoot per pot was observed to flower. The high dead shoot frequency observed for all SD treatments may explain the low flowering occurrence.

Other factors such as high temperature, light intensity or size of pot might be among the stresses promoting shoot death. Figure 4.4 also shows that pots with high numbers of shoots showed the highest number of dead shoots; therefore, the number of shoots per clump at the start of SD could also play a role. Since heliconias are clump plants, the number of shoots in the clump, or plant competition, could affect flowering. A negative linear relationship between number of shoots and inflorescences was reported in *H. angusta* by Kwon (1992). A dense population of shoots may have caused severe competition among the pseudostems resulting in insufficient amount of assimilates for full flower development. The hypothesis of the clump effect was also suggested by the occurrence of flowering in all pots with low numbers of shoots. In banana, a heliconia relative, the selection of the sucker follower to synchronize and promote production was a horticultural practice. Lassoudiere (1980) reported that when all the suckers were allowed to grow, only two flowered, while the rest died.

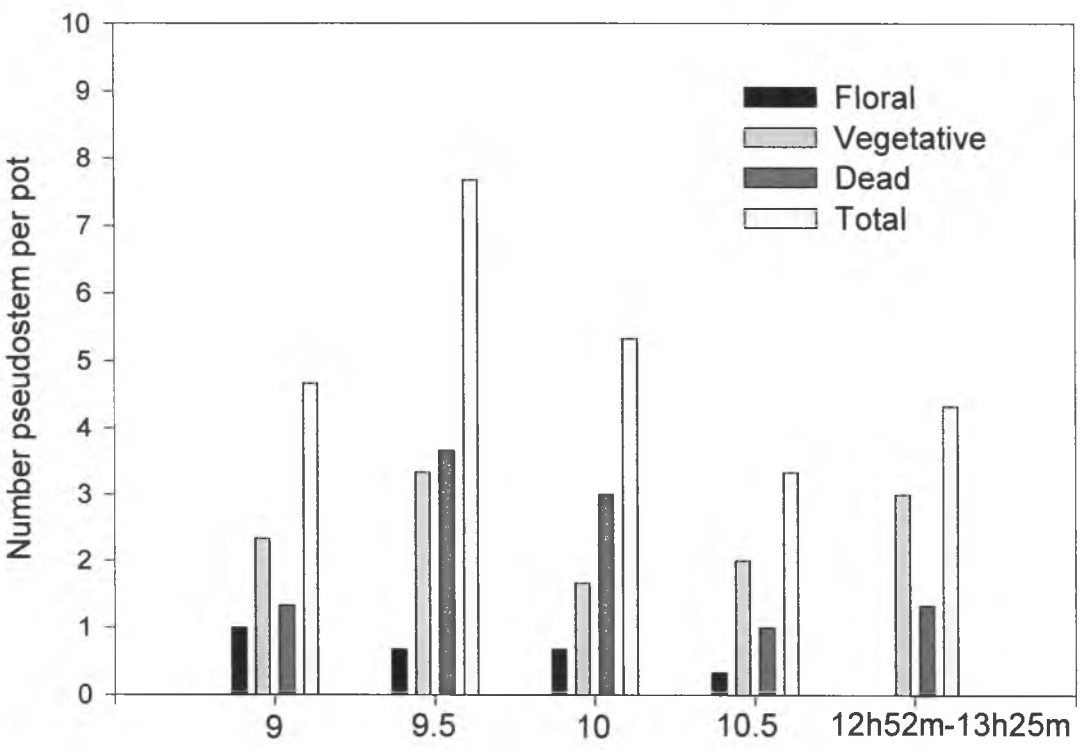


Fig. 4.4. Influence of daylength on the numbers of floral, vegetative and dead per pot 28 weeks after the onset of 8 weeks of short days

4.4.2. Effect of daylength and number of weeks (Exp. 2.)

Thirty-six weeks after the onset of the daylength treatments, a total of 465 shoots were counted. Among these 465 shoots, 90 flowered, 252 were still vegetative and 123 were dead. Flowering shoots represented 20 % of total shoots.

The reference plants grown under natural daylength during all phases of this experiment flowered during their natural season in Hawaii, while plants subjected to the different artificial daylengths began to flower earlier. The emergence of the earliest inflorescence was observed during the third week of December for plants given artificial SD while for plants under natural daylength, emergence occurred at the end of March.

The daylength treatments began July 15, 152 days before the first inflorescence appeared in the third week of December. The artificial daylength treatments were effective in inducing flowering, allowing the plants to flower almost three months before the natural flower season. The period of inflorescence emergence for the SD plants extended from the third week of December to the last week of January. The time period between the onset of the treatments and the emergence of the inflorescence (22 weeks) was similar the results in the previous experiment (21 weeks). In both experiments inflorescence emergence occurred over a period of 7 weeks.

Flowering occurred in all treatments. No significant differences in percentage of flowered pots were detected among the artificial daylength treatments (Appendix Table IV.i). However, it is possible to observe in Figure 4.5 that the percentage of flowering pots grouped by daylength treatment was higher (80 %) at 11.5 hours than at 9.5 and 10.5 hours (67 and 63 % respectively). Figure 4.6 shows that the percentage of flowered pots increased from 50 %, under 4 weeks of inductive daylength, to 83 % at 7 or 8 weeks. The

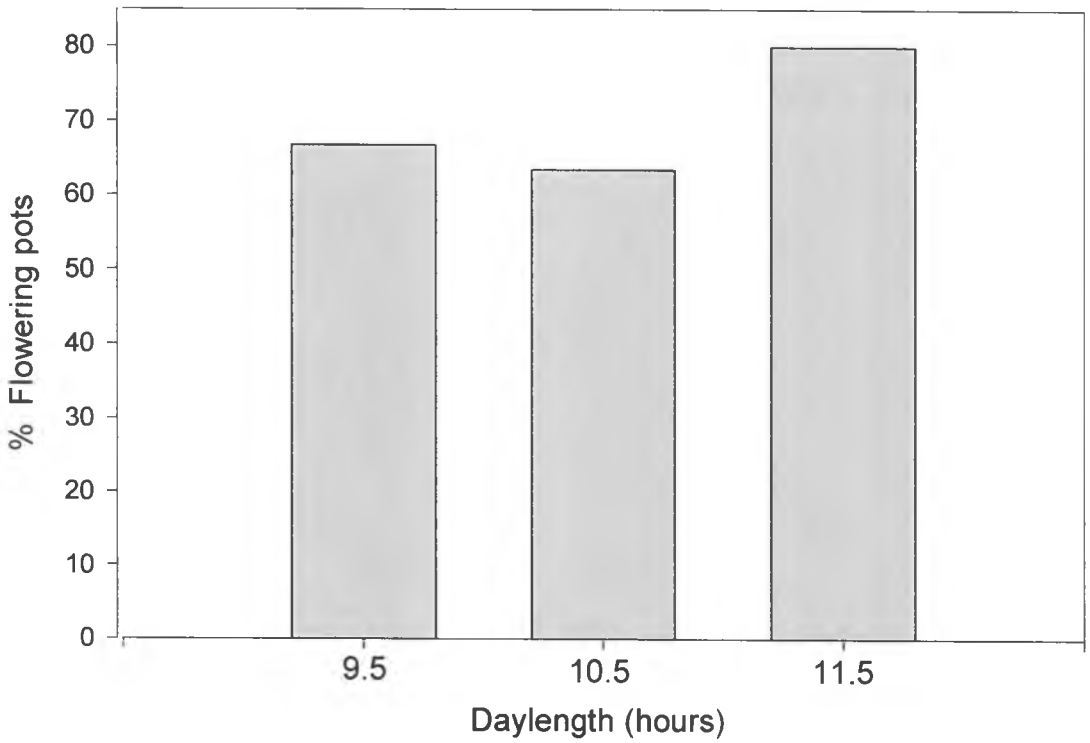


Fig. 4.5. Distribution of flowering as percentage of flowering pots by daylength in *H. rostrata*

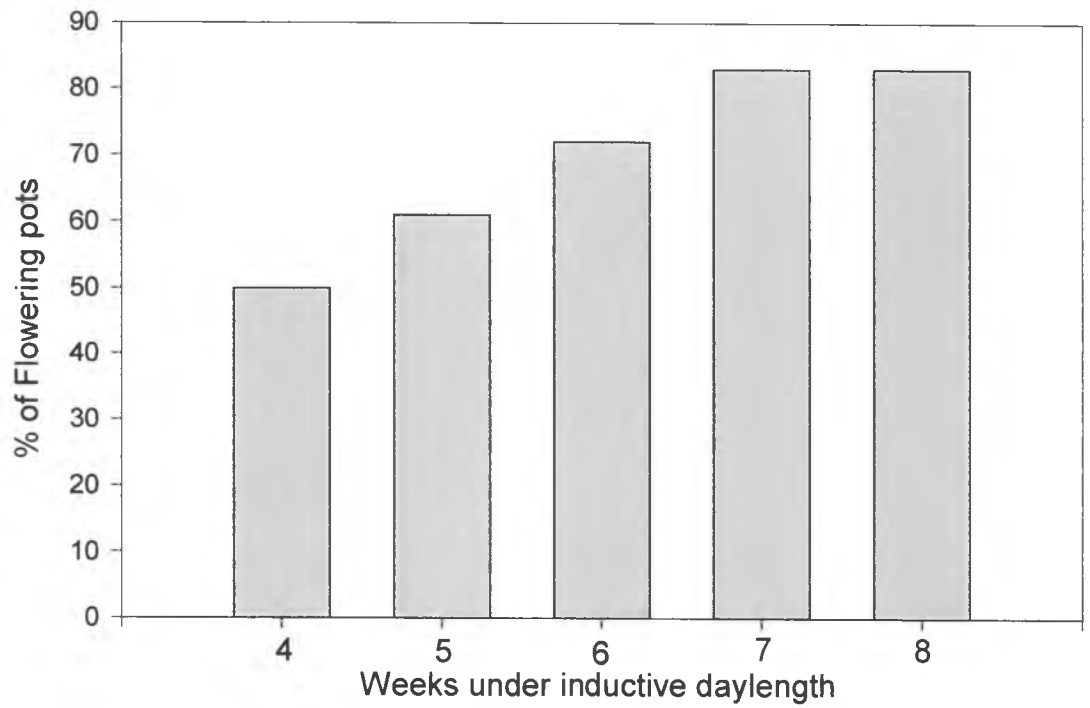


Fig. 4.6. Distribution of flowering as percentage of flowering pots by number of weeks under induction in *H. rostrata*

effects of the combination of treatments for the percentage of flowered pots are shown in Figure 4.7. The larger number of inflorescences corresponded to the inductive period of seven weeks under 10.5 hour daylength followed by 7 or 8 weeks under 11.5 hour and 8 weeks under 9.5 hours. Even though all the daylength treatments used induced flowering, 11.5 hour of daylength for 5 or more weeks showed a general trend to induce larger amount of inflorescences; while that the least effective treatments were 9.5 and 10.5 hours per period of 4 and 5 weeks.

These results suggested that the increasing of flower shoots with the increase of daylength or number of weeks under inductive treatments as result of increasing number of shoots been competent to floral induction rather than a effects of the treatments per se.

Even though the critical daylength and number of weeks for flower initiation were not established in this experiment the results suggest to use 11.5 hours daylength for more than 6 weeks to induce highest number of blooming shoots in this species.

The factors (high temperature, crowding, etc.) described in the previous experiment (4.3.1) affecting the flowering could also apply for this experiment. However, since in several short-day rice cultivars, flowering was reduced when the day was extremely short or when the light period was given at low irradiance (Ikeda, 1985), the lower percentage of flowering observed under the shorter daylengths could be a consequence of low assimilates in the plant.

Since the number of inflorescences per pot was variable between pots, Figure 4.8 shows the combination of daylength and number of weeks under inductive treatments on the number of flowered inflorescence. Similar conclusions can be drawn from this to express the results.

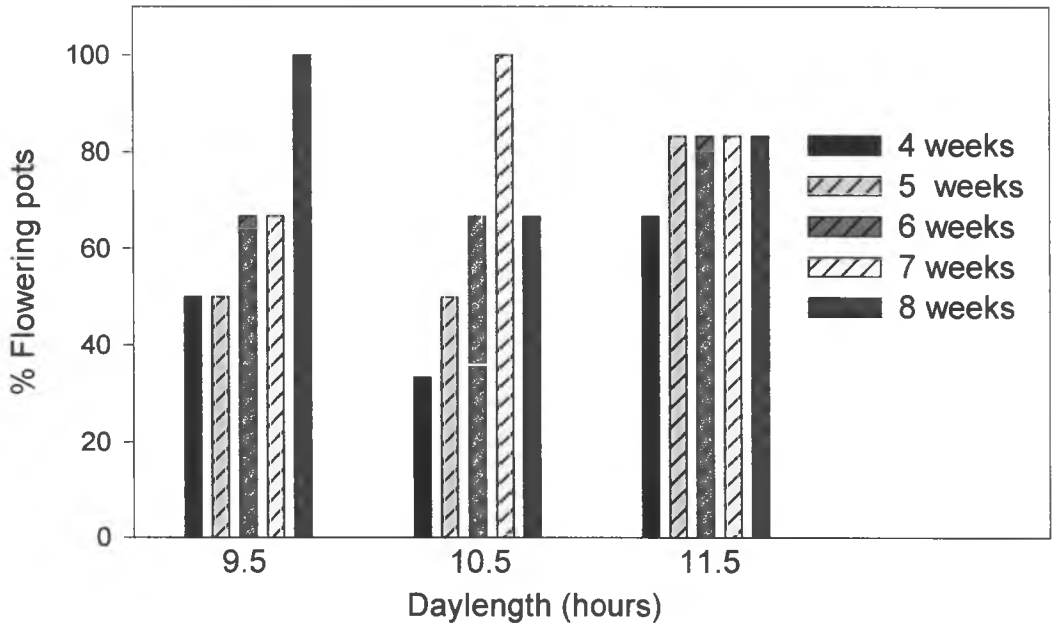


Fig. 4.7. Effect of daylength and the number of weeks under treatment on the percentage of flowering pots of *H. rostrata*

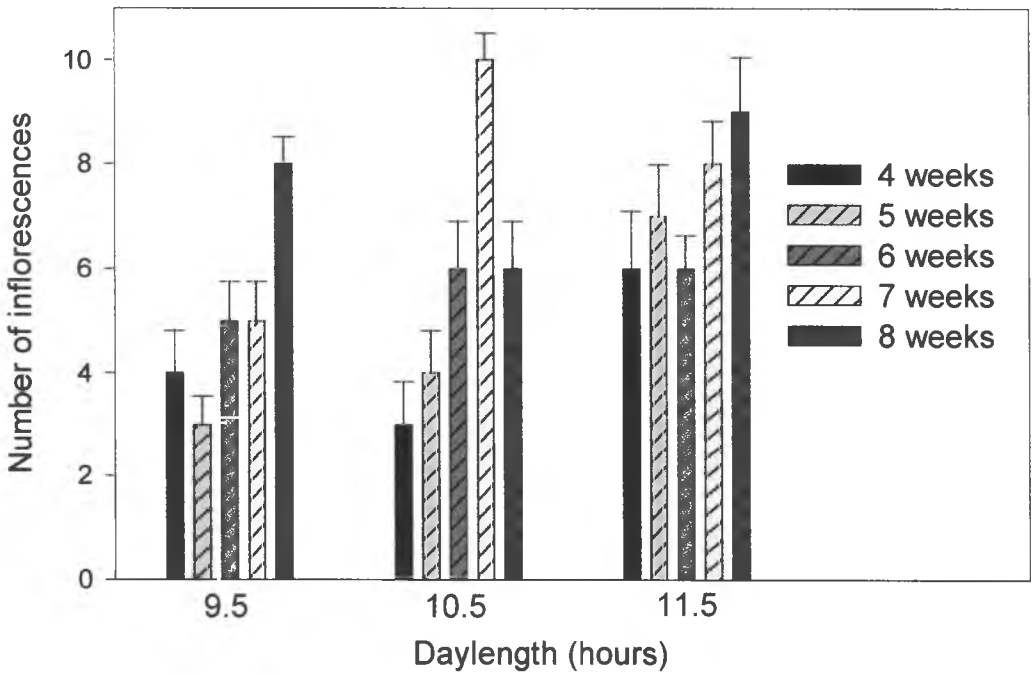
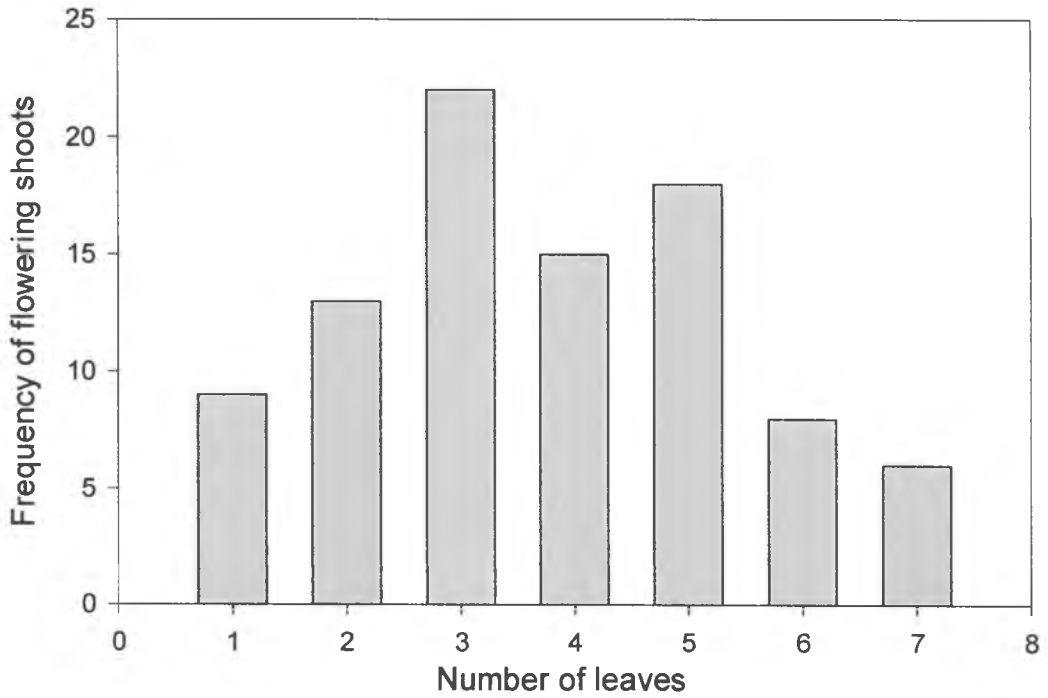


Fig. 4.8. Effect of daylength and number of weeks under treatments on the number of inflorescences of *H. rostrata*

Figure 4.9 shows the distribution of flowered shoots per number of leaves at the onset of induction. The highest flowering corresponded with shoots having from 3 to 5 leaves at the start of SD, although shoots from 1 to 7 leaves also flowered. In shoots with more leaves at the onset of treatments, the flowering occurred mainly when the number of weeks under inductive period was up to 6 weeks. When shoots with 2 leaves under 5 weeks of treatment flowered, other older shoots in the clump flowered too. Since it is unknown if the induction can be translocated from an induced shoot to another shoot in the clump, the minimal number of photoperiodic inductive weeks at the younger stage of shoot development (competent stage) can not be deduced. But, in competent shoots 4 weeks under short days was enough to induce flowering.

The relation between the number of leaves per shoot at the onset of induction and the flowering state is shown in figure 4.10. The number of leaves that subtend the inflorescence was directly correlated (R^2=0.768) with the number of leaves at the onset of induction (Appendix Table IV.ii). Shoots with 1 and 2 unfurled leaves at the onset of SD expanded an average of 5.8 new leaves by inflorescence emergence. While the shots with 4 or more leaves emitted from 5 to 5.2 leaves. Shoots with 3 unfurled leaves added 5.5 new leaves. Since, the shoot requires a minimum of three leaves to be competent for induction, the variation in number of leaves should be explained by the extra leaf (0.8) that a non competent shoot (example shoots with 2 expanded leaves) need to be competent (for example shoots with 4 expanded leaves). The sensitivity seems to be similar among competent shoots.



4.9. Frequency distribution of floral shoots per number of leaves at the onset of induction on *H. rostrata.*

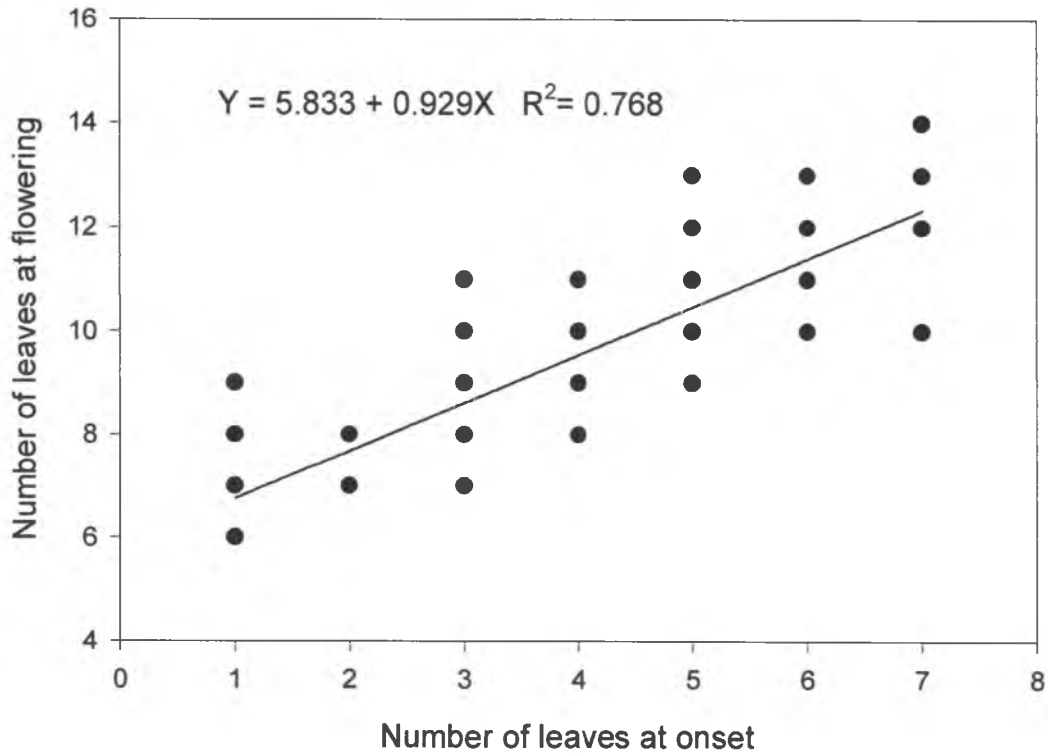


Fig. 4.10 Relation between the number of leaves per shoot at the onset of induction and at the floral state in *H. rostrata.*

The number of leaves that the shoots added after the onset of SD (from 5 to 5.8 leaves) in this experiment is slightly higher than the number of leaves found covering the reproductive apex of shoots sampled under natural inductive conditions (Chapter 3.4). This could be due to the time of sampling. Under natural conditions the number of leaves was determined in shoots already reproductive, while in this experiment the leaves were counted from the onset of SD.

The flowering of shoots with 1 leaf at the beginning of SD can be explained by the number of weeks under SD and the unfurling leaf rate. Similar results for the number of weeks of inductive photoperiod were discussed in previous experiment, since shoots with one leaf starting the SD had produced four leaves at the end of 8 weeks of SD. On the other hand, the rate of leaf unfurling increases with the leaf count in this species, since the leaf lamina area and pseudostem increase in size. Leaf emergence intervals from two to three weeks were required for second and third leaves, while fourth and fifth leaves took more than 4 weeks. In addition, in shoots with equal number of leaves at the onset of induction some variation may be expected due to differences in synchronization of growth. For example, some shoots received the inductive treatment just after the unfurling of the leaf and others when the following leaf was almost unfurled. The apical meristem was thus in different phases of primodium differentiation.

No significant differences were detected among treatments for the number of vegetative shoots (Appendix Table IV.iii). Similar numbers of vegetative shoots (82, 85 and 85) were observed under 9.5, 10.5 and 11.5 hours of daylength (Figure 4.11). They were also similarly distributed (from 48 to 53 shoots) along the number of weeks under

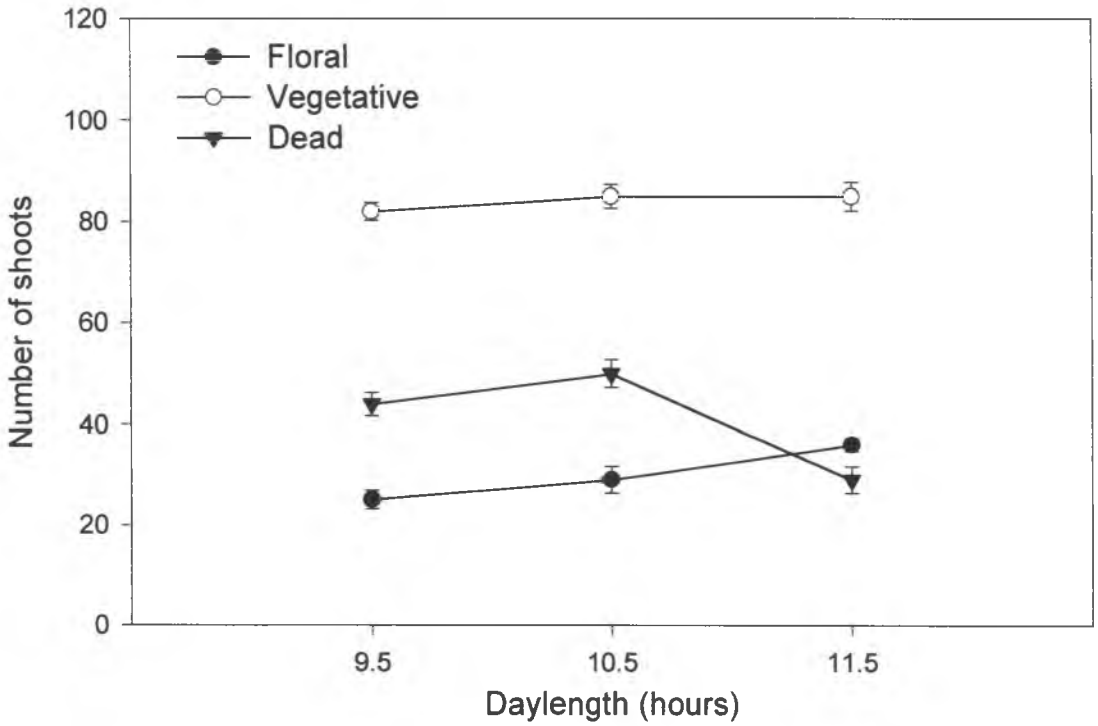


Fig. 4.11 . Total shoots at the end of the experiment by status and daylength on *H. rostrata*

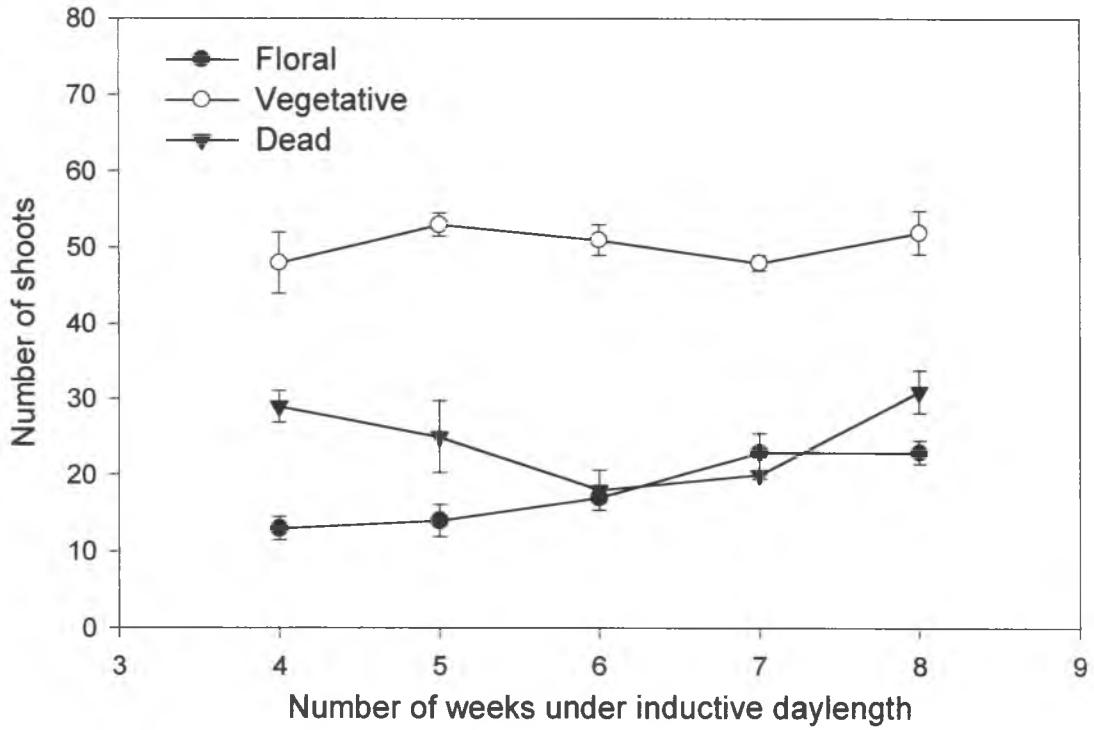


Fig. 4.12. Total of shoots at the end of the experiment by status and number of inductive weeks in *H. rostrata*

treatment (Figure 4.12). The vegetative shoots were mainly developed after the onset of the treatments. Nevertheless, 38 of them had already more than three leaves and did not flower. This category represented 15 % of the vegetative shoots counted at the end of the experiment, and 8 % of the total shoots. Many of these shoots with potential to flowering had five or six leaves at the onset of the daylength treatment, while their leaf number varied between eight and fourteen at the end of the experiment. The number of dead shoots at the end of the experiment was high, representing 26 % of the total shoots, a value higher than the flowered shoots (20 %). Although no significant differences were detected (Appendix Table IV.iv), the number of dead shoots was lower in plants that were induced under 11.5 hours daylength than in the ones under 9.5 and 10.5 hours (Figure 4.11). The period of induction also did not show significant differences, however, fewer dead shoots were observed for inductive periods of 6 and 7 weeks (Figure 4.12). Shoot death numbers were similar for 4, 5 and 8 weeks of SD.

Among the 123 dead shoots, 101 of them were tagged at the onset of the treatments. Eleven died in the first 2 weeks following the beginning of the treatments, since no more unfurled leaves were labeled after that (Figure 4.13). Fifty-five of the shoots failed to produce new unfurled leaves between weeks 10 and 15. The remaining 34 died between weeks 20 and 25. Figure 4.14 shows that among the dead shoots, there were individuals with different leaf numbers.

These results suggest two important periods when the unfurling of new leaves stopped. The most important was observed between weeks 12 and 15 after the onset of the inductive photoperiod, and the other one between the weeks 20 and 25. Because this

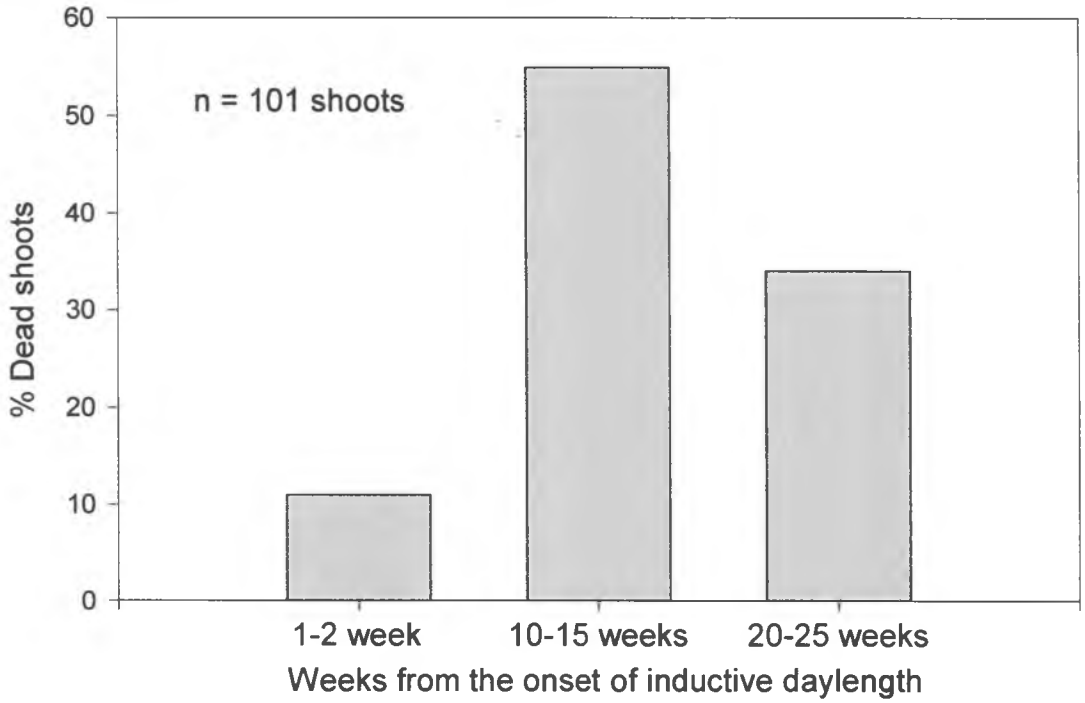


Fig. 4.13. Percentage of dead shoots by the period when no more more leaves expanded in *H. rostrata*

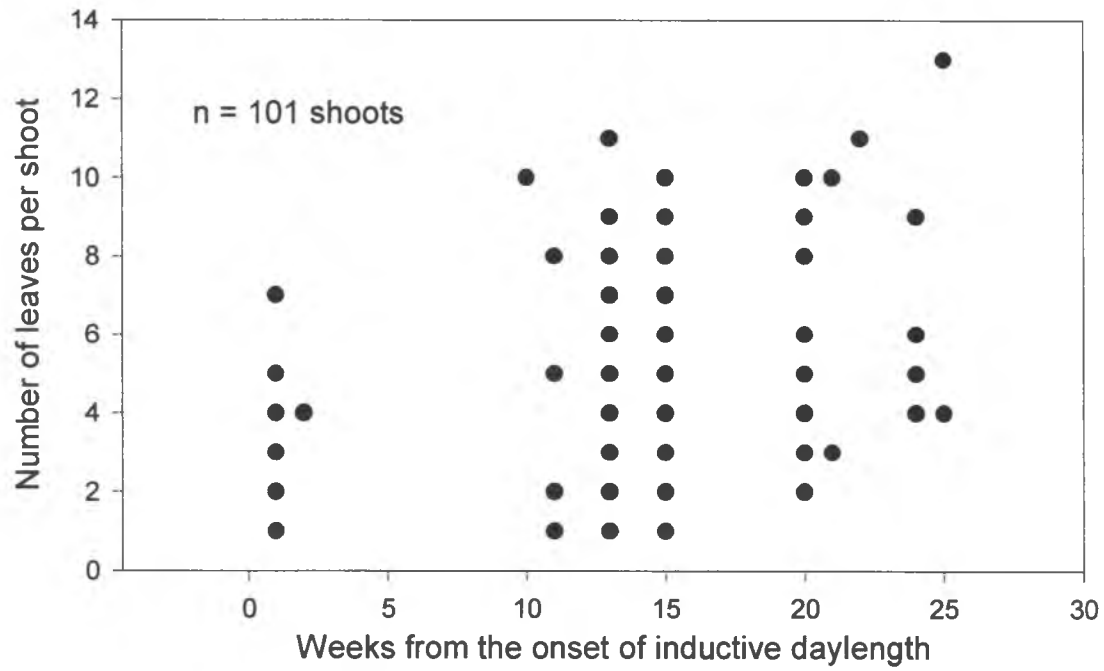


Fig. 4.14. Relationship between the number of leaves for dead shoots and the period when no more leaves expanded in *H. rostrata* from the onset of SD to flowering

determination was at the cessation of leaf appearance (external), it could not represent the critical period of development when the death of the apex occurred. Differentiated leaves could continue to expand after the death of the apex. Dead shoots detected during the two weeks after onset of treatments could be shoots already dead. On the other hand, the number of leaves per shoot seems not be a factor promoting death since it occurs in shoots with different leaf number.

If it is accepted that leaves already formed and more advanced in development at the apex (3 or more) will continue growing, then the first period, when no new leaves were produced by the apex, could be a consequence of no more leaf formation after the induction treatment. Apical death might occur during induction. However, there is possible that apical death occurred during the process when the apex is forming the structures of the inflorescence. It is known from the previous experiment (Figure 4.2) that shoots that will flower unfurl the leaf preceding the inflorescence (last or "flag" leaf) about two to four weeks before the emergence of the inflorescence from the pseudostem. Dissection of apices from shoots subjected to 9.5 hours of daylength for 8 weeks, sampled from the weeks 6 to 12 after the onset of SD, did not show flower differentiation. Lekawatana (1995) reported for *H. stricta* 'Dwarf Jamaican' that apex death is a consequence of flower abortion during its formation in the inflorescence.

The second period of shoot death with no leaf appearance might be explained by the different stages of development of the shoots in the clump over 8 week period of induction. Shoots that were induced later may have exposed the inflorescence later at weeks 20-25.

4.4.3. Effect of temperature and daylength (Exp. 3.)

A total of 482 shoots were counted at the end of the experiment. Their numeric and percentage distribution by daylength and temperature is shown in Table 4.1. The highest number of shoots (178) was found at 9 hours daylength and the lowest (145) at 11 hours. Whereas, by temperature, the highest number (184) was observed at 26 °C and the lowest (144) was a 21 °C. But, only 187 out of 482 total shoots had potential to be induced, since they had produced three or more leaves by the middle of the inductive period (at least four weeks under inductive treatment). These potential inductive shoots represent 39 % of the total number of shoots counted at the end of the experiment.

The ANOVA for the treatments (see also Table 4.1) detected significant differences for daylength ($P \leq 0.05$) and temperature ($P \leq 0.001$) on the parameter total number of shoots per pot (Appendix Table IV.v). No differences were found for the interaction of both factors (daylength and temperature). When the number of shoots per pot with potential to flower was statistically analyzed by daylength and temperature significant differences were only detected for temperature ($P \leq 0.05$) (Appendix Table IV.vi).

Figure 4.15 shows the mean separation of the total number of shoots per pot and shoots per pot with potential to be induced by treatment. The highest mean for total shoots per pot by daylength occurred at 9 hours and the lowest at 11 hours (Figure 4.15A). The mean at 13 hours was statistically equal to the other two treatments ($P \leq 0.001$). Since plants under the shortest and longest daylength behaved statistically equal, it was not clear how daylength promoted this pattern of response. No differences

Table 4.1. Distribution of shoot totals and shoots with potential to be induced (number and percentage) of *H. rostrata* at the end of the experiment by daylength and temperature. There were 8 pots in each of the nine treatment combinations.

	Temperature			
Daylength	16 ° C	21 ° C	26 ° C	Subtotal
	Total of shoots			
9 Hours	53 (11 %)	55 (11.4 %)	70 (14.5 %)	178
11 Hours	46 (9.5 %)	44 (9.5 %)	55 (11.4 %)	145
13 Hours	50 (10.4 %)	45 (9.3 %)	64 (13.3 %)	159
Subtotal	149	144	189	Total 482 (100 %)
	Shoots with potential to be induced			
9 Hours	19 (10.2 %)	20 (10.7 %)	23 (12.3 %)	62
11 Hours	21 (11.2 %)	20 (10.7 %)	24 (12.8 %)	65
13 Hours	20 (10.7 %)	16 (8.6 %)	24 (12.8 %)	60
Subtotal	60	56	71	Total 187 (100 %)

Significance for number of shoots per pot

	Total	Potential
Daylength	*	NS
Temperature	***	*
Daylength x Temperature	NS	NS

NS, * , *** Nonsignificant or significant at $P \leq 0.05$, $P \leq 0.001$, respectively.

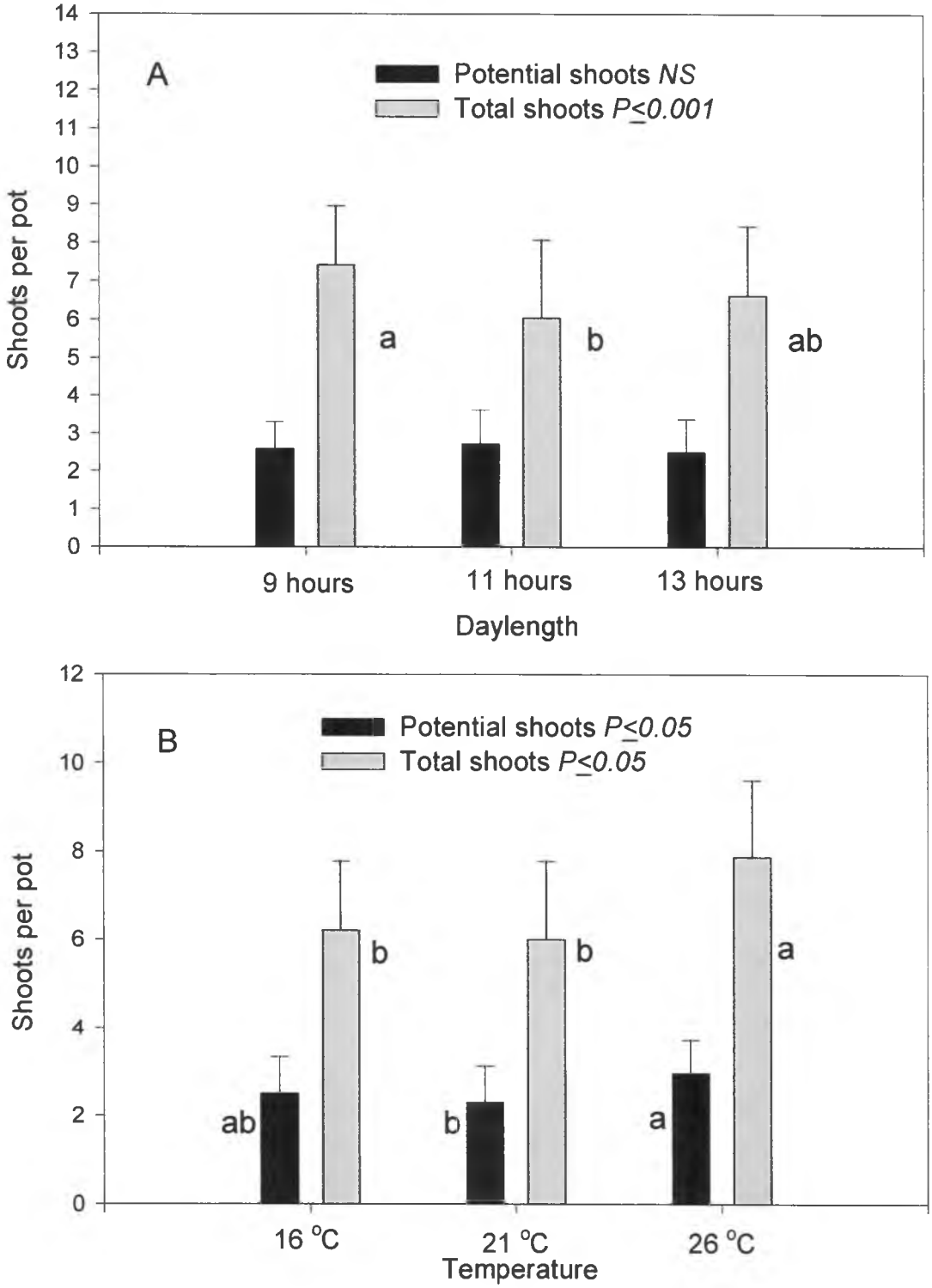


Fig. 4.15. Total number of shoots and shoots with potential to be induced per pot in *H. rostrata* by daylength (A) and temperature (B). Vertical bars are standard deviations, with mean separation (a,b) within shoot determination by Bonferroni multiple comparisons.

within daylength treatments for the number of shoots with potential to be induced were detected. Figure 4.15B shows the means of total number of shoots and shoots with potential to be induced according to night temperature during inductive SD. The most shoots, both total and potential, were found at the highest night temperature (26 °C); while the lowest number was observed at 16 and 21 °C for total number of shoots ($P \leq 0.05$) and at 21 °C for the potential shoots. These results showed that the lowest temperatures during the eight-week period of induction (8 weeks) decreased the total number of shoots developed during the experiment. Even though less clear a similar trend was observed for potential shoots. The time required for the development of the shoots might explain why differences were not observed for daylength in the potential shoot parameter.

Plants grown under 9 and 11 hours of daylength at all temperature treatments did flower. No flowering occurred for plants grown under 13 hours of daylength. ANOVA detected statistical difference ($P \leq 0.01$) for the number of inflorescences per pot among the daylength treatments (Appendix Table IV.viii). No differences for inflorescences per pot were detected for temperature or the interaction daylength x temperature. Figure 4.16 shows the means of the flowered shoots per pot by daylength. The treatments of 9 and 11 hours of daylength were statistically equal ($P \leq 0.05$).

The results proved that night temperatures between 16 and 26 °C were unable to induce flowering when the daylength was 13 hours. Thus the effect of decreasing temperatures progressively nullifing the daylength requirements reported for absolute SD and LD plants in other species ((Bernier et al, 1981a) was not observed in *H. rostrata*. This species was dependent on a shorter daylength to be induced to flower.

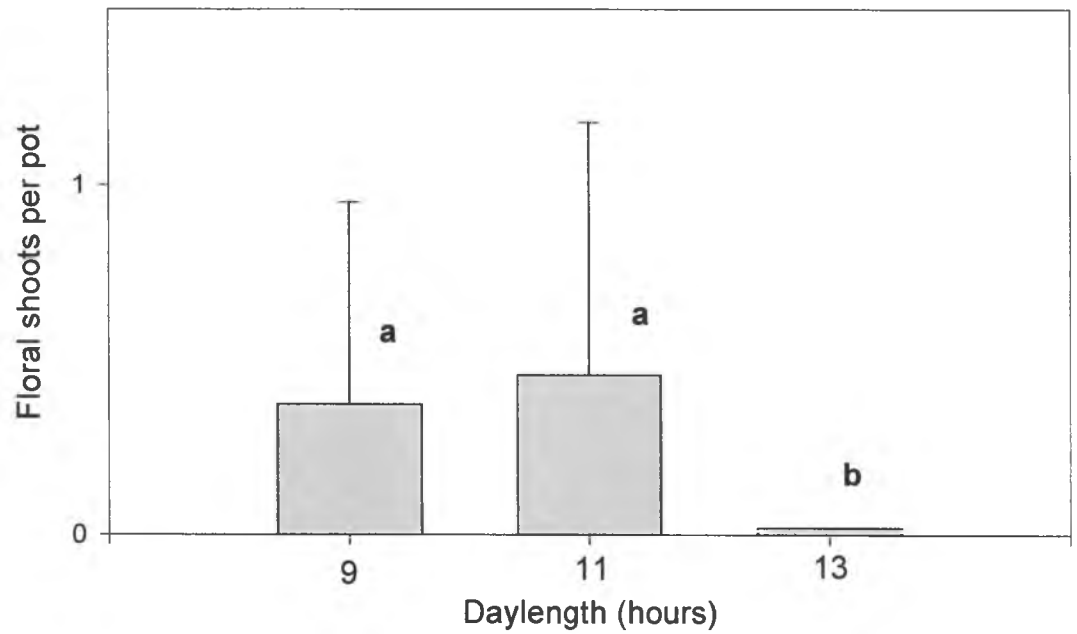


Fig. 4.16. Floral shoots of *H. rostrata* per pot by daylength. Vertical bars are standard deviation, with mean separation (a,b) by Bonferroni multiple comparisons ($P \leq 0.05$).

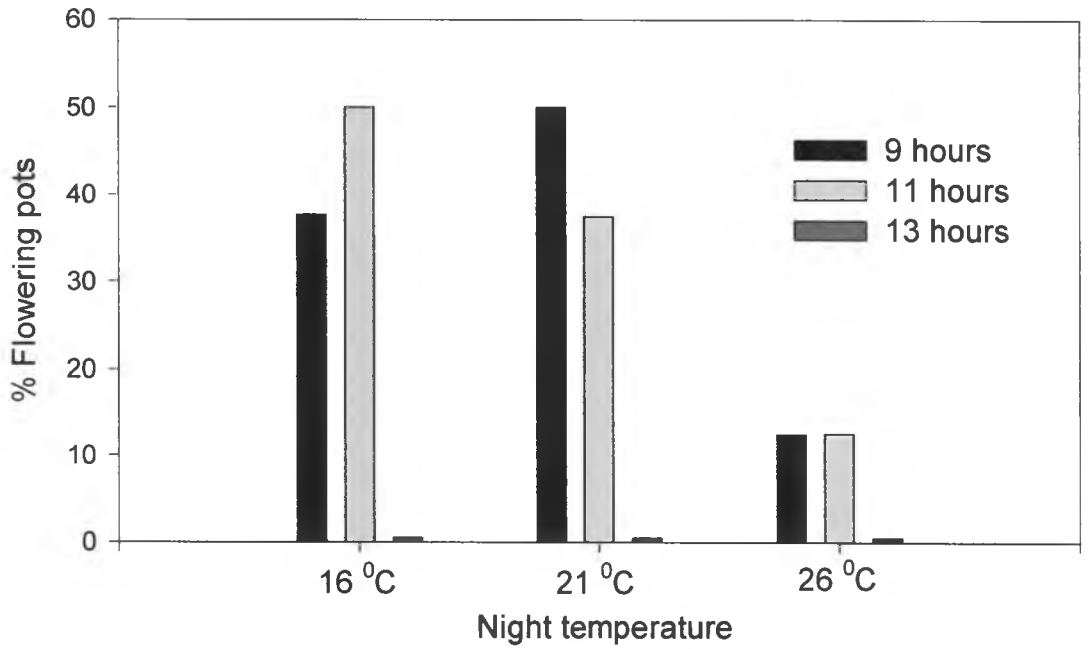


Fig. 4.17. Percentage of flowering pots of *H. rostrata* at different daylengths and temperatures,

The percentage of flowered pots (Figure 4.17) at 16 and 21 °C at night (37.5 to 50%) was higher than at 26 °C (12.5 %). The decreased flowering with increasing night temperatures during the inductive period was similar to the results reported by Lekawatana (1986) for *H. stricta* 'Dwarf Jamaican', which decreased from 53 % to 14 % as night temperatures increased from 15 °C to 25 °C.

Temperature was not an inductive flowering factor of *H. rostrata* per se, but temperatures during the induction modified the percentage of floral shoots. This modification could be a consequence of temperature as a factor affecting the sensitivity to be induced and/or the rate of growth. High night temperature, at the start of SD, delays the onset of flower initiation in chrysanthemum, a SD plant (Cockshull and Kofranek, 1994). On the other hand, high temperatures can accelerate the growth of the shoots therefore promoting a higher number of inducible shoots, which could lead to more competition between shoots for assimilates and increase shoot death.

The emergence of the earliest inflorescence was observed during the third week of May, 24 weeks after the onset of the treatments. The period of inflorescence emergence extended for nine weeks from May 17 to July 19. The earliest inflorescences occurred in both (9 and 11 hours) daylengths and lower night temperatures (16 and 21 °C). Figure 4.18 shows the percent distribution of inflorescences by time from the onset of the treatments. The highest percentages of inflorescences emerged in weeks 24 (35 %) and 29 (20%), four weeks apart from each other.

The time required from induction to appearance of earliest inflorescences (week 24) and the period between the earliest and the last inflorescences (9 weeks later) were similar to the previous experiments (the earliest at weeks 21 or 22 weeks and the last 7

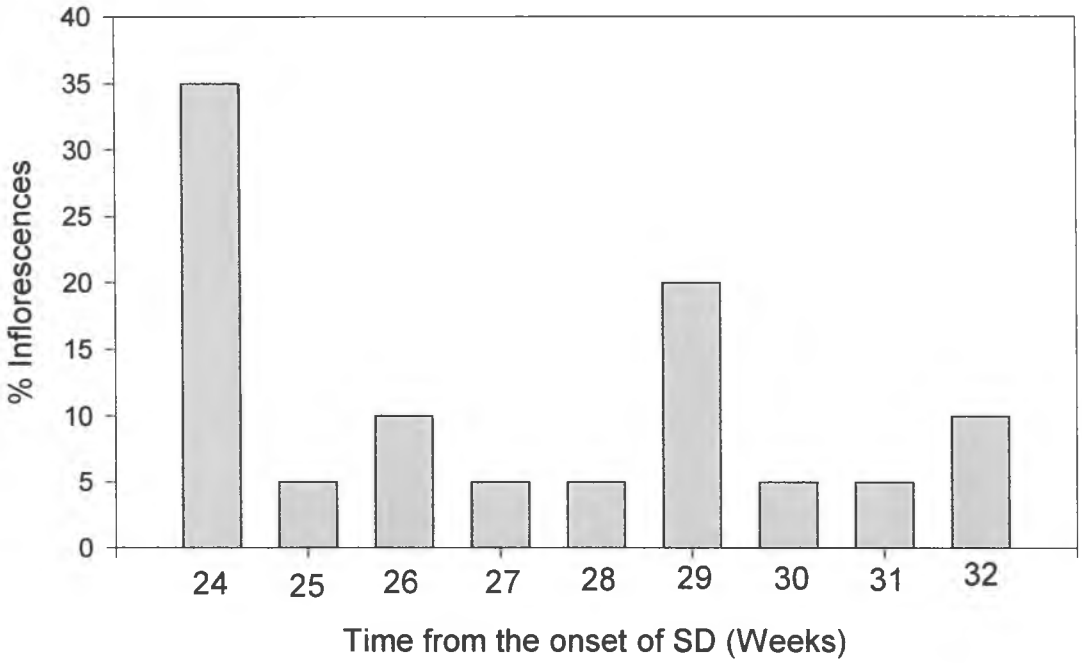


Fig. 4.18. Frequency of *H. rostrata* emergences from the onset of SD expressed as a percent of total inflorescence production (n=20). Temperature treatments were pooled by time from the onset of SD.

weeks later). Two additional weeks could be attributed to shoot stage, environmental conditions, interval of tagging and/or biological variation. However, since the period of inflorescences emergence was as broad (9 weeks) as the number of weeks under induction (8 weeks), no conclusive effects of temperature in advancing or delaying flowering could be derived from these observations.

Figure 4.19 shows the final distribution of shoots with initial potential to flower by status (flowered, vegetative and dead), daylength and temperature. ANOVA detected statistical difference ($P \leq 0.01$) for the total number of inflorescences only for daylength treatments (Appendix Table IV.viii). Both 9 and 11 hours of daylength were statistically equal, but different from 13 hours (Bonferroni multiple comparisons, $P \leq 0.05$). No differences were detected among the treatments for the number of vegetative shoot (Appendix Table IV.ix) or for the dead shoots (Appendix Table IV.x). However, a lower total number of vegetative shoots (Figure 4.19 B) and a higher number of dead shoots (Figure 4.19 C) were observed when the daylengths were 11 hours and 13 hours independent of the temperature. Also, in general, the highest flowered shoots (11 hours) corresponded with the fewest vegetative shoots and fewer dead shoots. On the other hand, an opposite relation was observed between vegetative and dead shoots when not flowering was present at 13 hours daylength and between flowered shoots and dead shoots for plants under inductive daylength (9 and 11 hours).

The number of dead shoots per treatment was higher in this experiment than in the previous ones. This might be attributed to environmental conditions such as the day air temperature average 37 °C (30 - 42°C) that was higher in the glasshouse during the last phase of this experiment. Also, since the glasshouse was not painted and the light

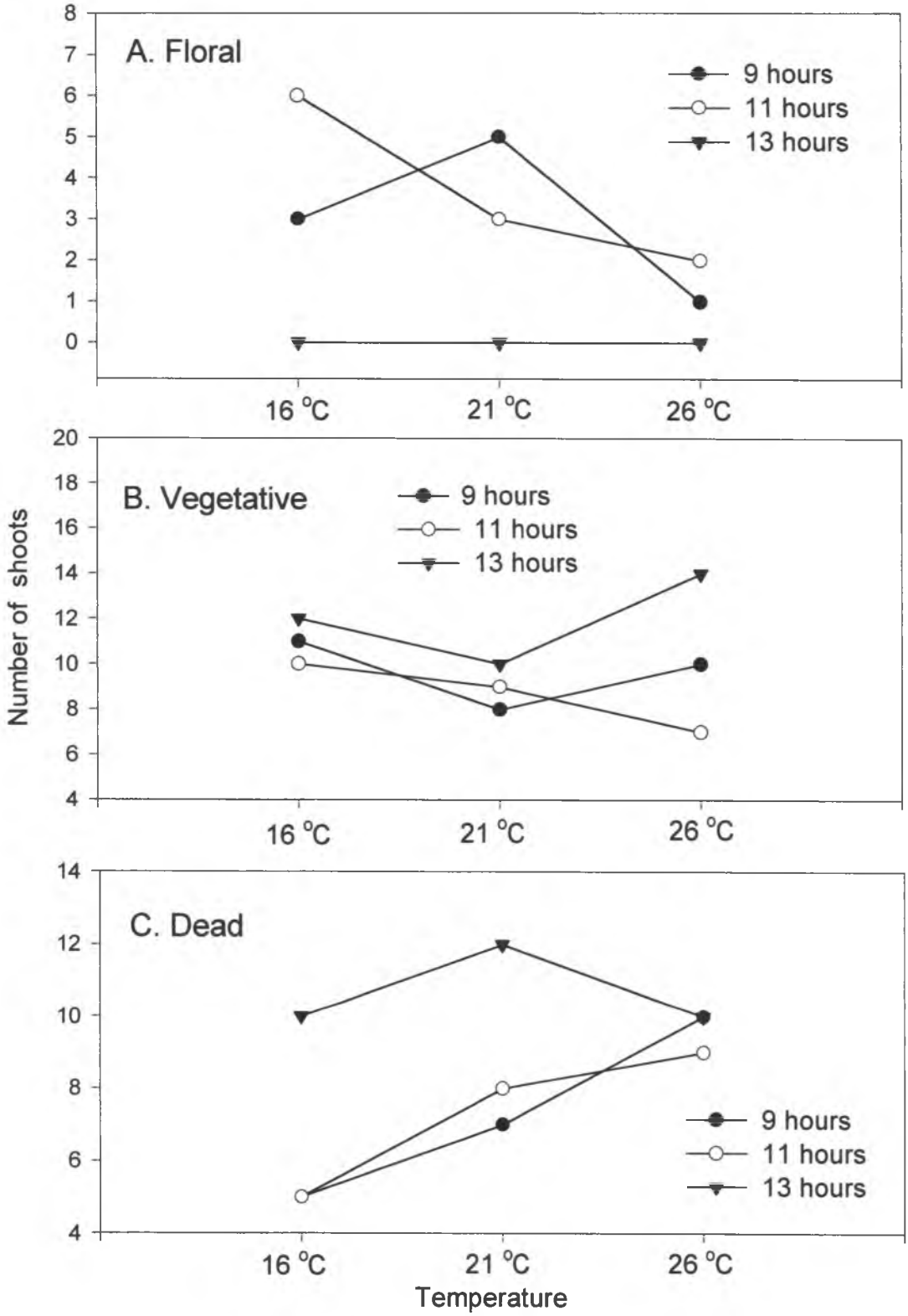


Fig. 4.19. Shoots of *H. rostrata* with potential to be induced by status (floral, vegetative and dead), daylength and temperature. Each data point represent the total from 8 pots.

intensity was high this may have been the stress factor that alone or together with high temperature promoted death of the apex. Photoinhibition was reported at high intensities for this species (He et al., 1996)

ANOVA detected significant differences among the total of vegetative shoots and vegetative shoots developed after the inductive treatment for daylength ($P \leq 0.01$) and temperature ($P \leq 0.001$). The highest numbers of total and after-treatment vegetative shoots were found in plants subjected to 9 hours of daylength (Figure 4.20A), while the lowest number corresponded to plants under 11 hours. The number of shoots at 16 and 21 °C were statistically equal but lower than at 26 °C (Figure 4.20B) (Appendices VI.xi and VI.xii).

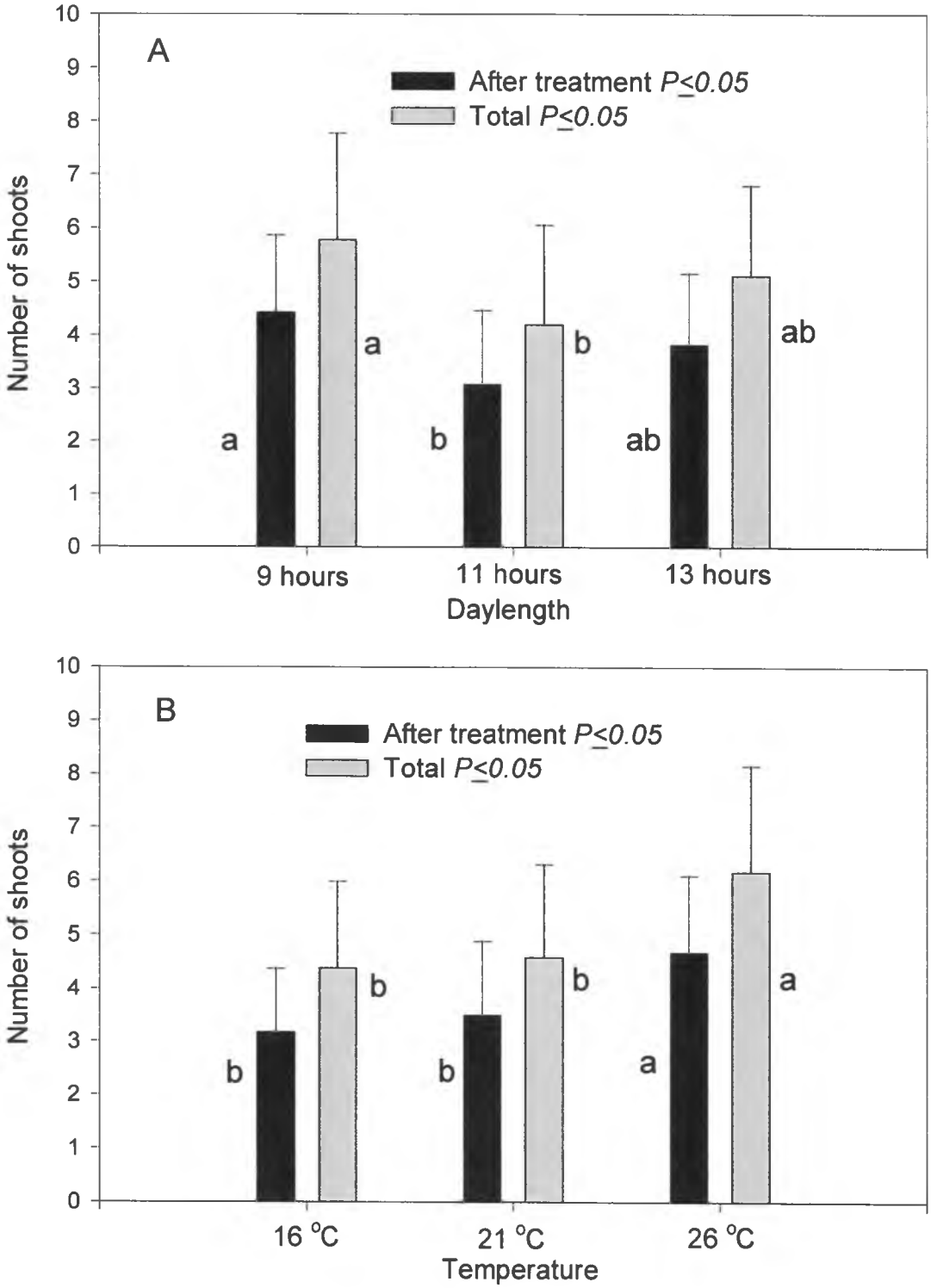


Fig. 4.20. Number of shoots developed after treatments and total of shoots in *H. rostrata* by daylength (A) and temperature (B). Vertical bars are standard deviations, with mean separation (a, b) within shoot determination by Bonferroni multiple comparisons.

4.5. Conclusions

Flowering in *H. rostrata* was advanced by imposing daylengths less than 11.5 hours long for at least 4 weeks. This species behaved as short day plant. The inflorescences emerge more than 22 weeks after the onset of treatments. The minimal number of leaves that the shoot required to be induced was three. Moreover, an important number of potential flowering shoots was still vegetative, and a high number of shoots died. The factors that induced death in the shoots were unknown but may be related to assimilate competition among shoots.

Night temperatures from 16 to 26 °C during inductive SD had no effect on the induction of flowering in plants grown under daylengths less than the critical daylength. No interaction effects between temperature and daylength affecting flowering induction were observed. But, night temperature during induction affected the total of shoots developed. Temperature might play a role in the sensitivity of shoots to be induced to flower and/or promoting competition for resources, since it affects the growth rate of shoots. At high night temperatures more shoots were developed which could promote the competition for resources to support the inflorescence. However, the high temperatures after induction might have increased shoot death in the last experiment.

CHAPTER 5
EXTENDED DAYLENGTH AND NIGHT INTERRUPTION IN THE INHIBITION OF FLOWERING OF *HELICONIA ROSTRATA* RUÍZ & PAVÓN

5.1 Abstract

The effects of extended daylength and night-break in the inhibition of flowering of *H. rostrata* were studied in three experiments. Flowering of potted plants, grown in glasshouses, was inhibited during their natural season (March to July) when they were under supplemental light (5pm to 10 pm) with incandescent bulb lamps from September 21, 1997 to February 1, 1998. The flowering was delayed until July, 24 weeks after the plants were returned to natural daylength. The daylength between February 1 and March 15 was short enough (from 11 h 31 m to 12 h) to induce flowering of plants having three or more leaves by early February. The critical daylength was between 11h 45m and 12h.

Flowering of plants exposed to night break (11.00 pm to 3.00 am) or supplemental light (5:30 pm to 9:30 pm) from December 1, 1998 to May 15, 1999 was also inhibited. Plants under natural short-days (control) flowered. The flowering of control plants occurred from May to August. Seven to nine leaves per pseudostem subtended the inflorescence. Shoots under extended daylength were still vegetative and already had produced eight to fourteen leaves at the end of the experiment. Since the vegetative growth phase of *H. rostrata* was extended using either one of the daylength

methods (night break or supplemental light), it could be assumed that the transition to flowering was inhibited by light.

Plants subjected to eight weeks of artificially extended daylength (18 hours), followed by shorter natural long days (<13h 20'), beginning on July 13, behaved like the control plants under natural daylength without promoting earlier flowering. Thus, the flowering process depends on a maximum critical daylength and not on a change in growth patterns promoted by the alteration of daylength.

These results, and the ones discussed on the previous chapters, allow us to conclude that *H. rostrata* is an obligate or qualitative short day plant.

5.2. Introduction

A SDP is one that only flowers or flowers most rapidly with less than maximum number of light hours in each 24 hour period. Light and time are two essential components of the photoperiodic process in SDPs. Light acts to control the phase of photoperiodic rhythm and, also interacts with a specific phase of that rhythm to inhibit flowering, Time is measured in darkness and the critical factor for floral induction is a sufficiently long dark period or, as in the majority of short-day plant (SDP), a succession of such dark periods (Thomas and Vince-Prue, 1997).

On the other hand, night interruption (night-break) and supplemental daylength with artificial light will prevent flowering in many SDPs under inductive conditions. Inhibition of flowering by light is of great practical interest because it provides a means of controlling the duration of vegetative growth. A few minutes of night-break at low energy can be enough to prevent flowering in most SDPs. The effectiveness of night-

break is also dependent on the point in the dark cycle when the treatment is given (Thomas and Vince-Prue, 1997; Cockshull, 1984; O'Neill, 1992).

Even though it has received relatively little attention, there is the possibility that plants might respond to a change in the duration of daylength rather than to an absolute duration of darkness. The perception of lengthening versus shortening days or nights would enable plants to discriminate between spring and autumn when absolute daylength would give an ambiguous signal (Thomas and Vince-Prue, 1997).

Extended daylengths with supplemental light from incandescent bulbs has been experimentally used to induce flowering in *H. angusta*, a long-day species (Lekawatana, 1986, Kwon, 1992). In *H. wagneriana*, a SDP, daylength manipulation supplied as supplemental daylength (6:00 to 10:00 p.m.) and as night-break from 10:00 p.m. to 2:00 am) was found to inhibit flowering (Criley and Sakai, 1997). From a practical standpoint, night-break or extended-day could be used to inhibit flower induction in short-day (SD) heliconias and manipulate the inflorescence production when the natural flowering season is flooding the markets.

As has been discussed in the previous chapters, *Heliconia rostrata* is a species that flowers after a natural or imposed period of short days. However it is unknown if this species is an obligate or facultative species. The objective of the experiments described in this chapter was to determine if this is a species inhibited by light or induced by decreasing of daylength. During the first experiment, plants were grown under extended daylength until February 1 and, after that under natural daylengths to analyze the effect of light to extend vegetative growth or delay flowering. In a second experiment, the inhibition of flowering during the natural SD by night-break and supplemental light was

studied. In the third experiment, the possibility that this species responded to a change in the duration of daylength rather than to an absolute long duration of darkness was analyzed by subjecting the plants to extended daylength followed by shorter natural long days.

5.3. Materials and Methods

5.3.1. Supplemental daylength during short days (Exp.4). The experiment was conducted at the Magoon and Pope Laboratory facilities of the University of Hawaii at Manoa. On August 7, 1997, rhizomes of *H. rostrata* were planted into sixteen plastic pots (20 cm diameter x 16 cm depth). The potting mix was peat moss, perlite, composted red wood, and volcanic cinder in a 1:1:1:1 v/v ratio. The substrate mixture was amended with dolomite, Micromax (minor elements) and treble superphosphate at a rate of 6.0, 1.0, and 0.6 kg m^{-3}, respectively. Plants were placed in a shadehouse with 30 % shade provided by saran cover. The plants were irrigated twice daily with nutrient solution (200 N- 0 P- 233 K ppm).

The plants were transferred to a glasshouse where the daylength treatments were initiated in September 16. Two groups of 8 pots each were placed in separate compartments at the glasshouse. One group of plants was subjected to extended long-day until February 1. This treatment was supplied by providing supplemental light from 5 p.m. to 10 p.m.. The source of light was incandescent lamps of 100 watt (3.8 Wm^{-2}) placed 1.6 m above the pots. The second group of plants (control) was grown under natural daylength, which ranged from 12h 9m on September 21 to 11h 31m on February 1. After February 1, the daylength was increased. Daylength was 12 h on March 14. The

average day time air temperature was 32 °C ranging from 25 to 37 °C, while the average night air temperature was 21 °C (ranging from 18 to 22 °C).

Plants were spray watered daily and fertigated twice a week with 1000ml/pot of nutrient solution of 500 ppm N-P_2O_5-K_2O at the ratio of 20-20-20 during this experimental phase at Pope laboratory.

The new expanded leaves were labeled every two weeks after the beginning of extended daylength. The number of shoots per pot and their status (flowered, vegetative and dead) were determined at the end of the experiment by pseudostem dissection. The number of leaves per shoot was recorded.

5.3.2. Supplemental daylength and night break (Exp.5). The experiment was conducted at the Magoon facility of the University of Hawaii at Manoa. On August 1, 1998, rhizomes of *H. rostrata* were planted into 8-liter pots containing a potting mixture and amended as in the previous experiment (5.3.1). Plants were placed in a shadehouse with 30 % shade provided by saran cover. The plants were spray irrigated twice daily, with nutrient solution (200 N- 0 P- 233 K ppm).

On December 3, 1998, shoots with more than 3 leaves were cut off, and the plants were transferred to a glasshouse where the daylength treatments were applied. Three groups of 12 pots each were placed on three separate benches in separate compartments with black plastic curtains.

A daylength treatment in the form of night break was applied to one group of plants. The night break was supplied by providing 4 hours of light from 11.00 p.m. to 3.00 am daily from December 3 until May 1. During the same period of time, a second group of plants was subjected to extended daylength treatment in the form of

supplemental light (from 5.30 p.m. to 9.30 p.m.). The source of light was three 100 watt incandescent lamps (3.8 Wm^{-2}) placed 1.5 m apart and at a height of 1.6 m above the pots. In a third group, the control, the plants were allowed to grow under natural SD. The natural daylength ranged from 10h 50m on December 21 to 13h 26m on June 21. The average day time air temperature was 30 °C ranging from 26 to 35 °C, while the average night air temperature was 20 °C (ranging from 19 to 22 °C).

The expanded leaves were labeled at the onset of daylength treatments and every two weeks for each new expanded leaf. At the end of the experiment (August 99), the number of shoots per pot and their status (flowered, vegetative and dead) were recorded. The number of leaves per shoot, as well as pseudostem length, was also recorded.

5.3.3. Sequence Extended-natural long days (Exp.6). This experiment was conducted at the Magoon facility and the Pope laboratory at the University of Hawaii at Manoa. Rhizomes of *H. rostrata* were planted into 8-liter pots on March 17, 1999. The potting amended mix was as in the previous experiment. Plants were placed in a shadehouse with 30 % shade provided by saran cover. The plants were spray irrigated twice daily, with nutrient solution (200 N- 0 P- 233 K ppm). Plants were transferred to Pope laboratory glasshouse on May 17, 1999, where daylength treatments were supplied.

Two groups of 8 pots each were placed in two separate compartments in the glasshouse. One group of plants was subjected to an 8 week period (from May 17 until July 13) of extended long-day of 18 hours. This treatment was supplied by providing a total of 9 hours of supplemental light daily; 6 hours from 5:30 p.m. to 11:30 p.m. and 3 hours from 5:30 am to 8:30 am. The source of light was one incandescent lamp of 100 watt (3.8 Wm^{-2}) placed 1.6 m above the pots. After 8 weeks of treatment, the treated

plants were transferred to natural daylength conditions, where the second group of plants (control) was located. The natural daylength during May 17 to July 13 ranged from 13h 10m 17 to 13h20m. After June 21, the longest day (13h 26m), the daylength started to decline. The average day time air temperature was 34 °C ranging from 27 to 38 °C, while the average night air temperature was 22 °C (ranging from 19 to 23 °C).

The new expanded leaves were labeled weekly during the daylength treatments and every two weeks after that until November 1 (24 weeks after the onset of daylength treatments). The number of shoots per pot and their status (flowered, vegetative and dead) were observed through dissection in December 20. The number of leaves per shoot and the pseudostem length as defined in chapter 3 were recorded.

5.3.4. Data analysis

Data collected from all experiments were analyzed for variance, and mean separations were performed. Correlation and regression analyses of plant parameters against treatments and between variables were performed whenever appropriate. The statistical process was accomplished using the computer program SYSTAT 7.0® for Windows (SYSTAT, 1997).

5.4. Results and Discussion

5.4.1. Supplemental daylength during short days (Exp.5)

No statistical differences were detected between the plants cultivated under extended daylength (from 5 p.m. to 10 p.m.) and natural daylengths during the natural short days (September 21 to February 1) for the number of total shoots, vegetative shoots, reproductive shoots and dead shoots (Appendices Tables V.i to V.iv). However, there was a difference ($P \leq 0.01$) in the number of leaves per shoot (Appendix Table V.v). The

average number of shoots per pot was 7 ± 2.39 and 6.4 ± 1.85 for plants under extended daylength and natural daylength at the end of the experiment in August (Figure 5.1). The number of leaves per shoot was higher in plants under extended daylength (10.6 ± 2.07) than under natural short days (8.29 ± 0.95).

The number of leaves from shoots under natural daylength conditions was similar to the number found in the previous experiments where plants with 1 to 4 leaves (Fig 4.10) were forced to flower under artificial SD. The differences in leaves per shoot between the treatments of this experiment can be explained by the addition of leaves under the extended daylength prior to the onset of the natural SD (February 1) when the plants were induced to flower.

The flowering expressed as percentage of flowered pots (Fig. 5.2) was 50 % and 62.5 % for plants under extended daylength and natural daylengths, respectively. Plants under natural daylength conditions started to flower during the second week of March, while the ones under extended daylength did not flower until the third week of July. During the additional time (18 weeks) that plants under extended daylength took to flower, 2.3 extra leaves were produced in the flowered shoots. Flowering occurred 24 weeks after turning off the lights. A similar period (23 to 24 weeks), from the induction to the emergence of the inflorescence, was shown by plants in the previous experiments. These results confirm the inhibiting effect of light on flower induction and the time required from induction to emergence of the inflorescence.

At the conclusion of extended daylength or February 1, the natural daylength was 11 h 31 m. On plants given the extended daylength treatment, shoots with 3 or fewer leaves 4 weeks into the natural SD period (Feb.1-March 1) did not flower, while shoots

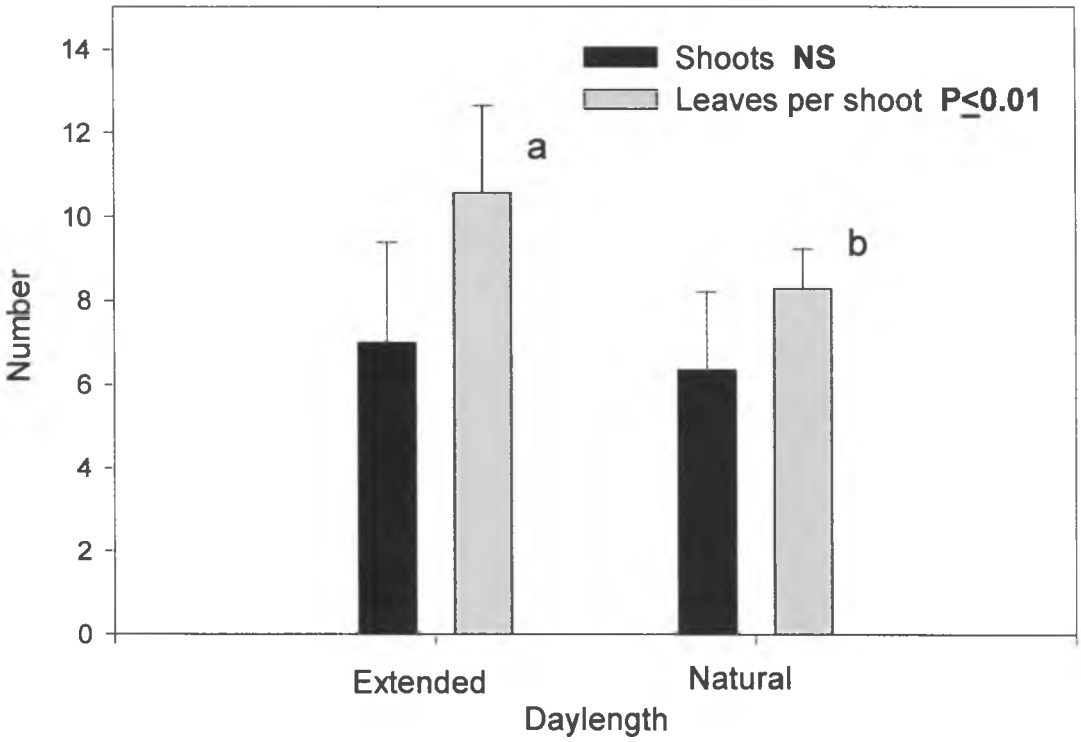


Fig. 5.1. Number of shoots per pot and leaves per shoot in *H. rostrata* under two daylength conditions (N= 8 pots per treatment).

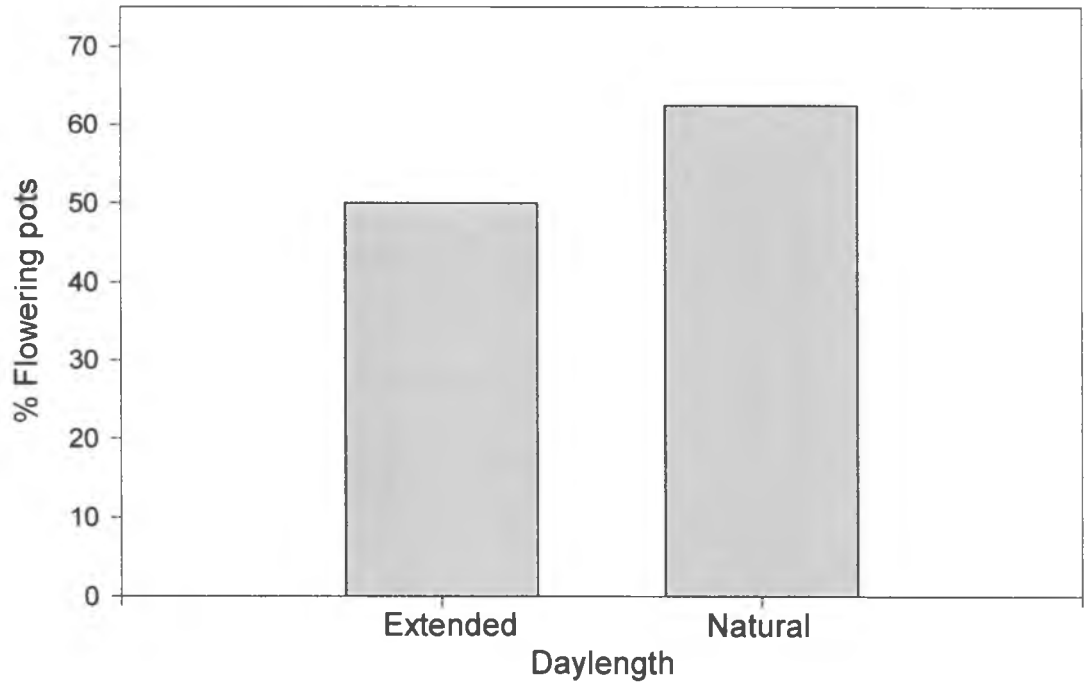


Fig. 5.2. Percentage of flowering pots of *H. rostrata* under two daylength conditions (N= 8 pots per treatment).

that had produced 4 leaves did flower. Since 4 weeks of inductive photoperiod is sufficient to induce flowering on 4-leaf plants, the critical daylength should be very close to the daylengths found between March 1 to 15 in Hawaii (11 h 44 m to 12 h 01 m). Figure 5.3 illustrates the period in Hawaii when *H. rostrata* shoots of 4 or more leaves can be induced.

Sharp critical photoperiods close to 12 hr have been observed in certain varieties of sugar cane and rice. When such plants are grown at low latitudes, they are able to perceive small changes in the daylength and exhibit seasonal flowering (Zeevaart, 1976). Since *H. rostrata* is native to the Equatorial region, where only small fluctuations in the daylength occurs throughout the year, it is not surprising that the critical daylength is close to 12 hours. At lower latitudes where daylength is homogenous during the year, a broader period of flowering is expected when the environmental conditions for growth are not limited. This may explain why the blooming in this species has been reported as year around (Berry and Kress, 1991; Castro, 1995).

Because the production of inflorescences tends to decrease at daylengths shorter than 11h 30m (Chapter 4) and the critical daylength lies between 11h 45m and 12h 00' a daylength of 11h 30m for 4 weeks should be used to induce flowering. The critical daylength is basic to refine strategies to manage the flower production. Flowering can be delayed by using artificial light to exceed the critical daylength. Even though this approach to control flowering is limited by the natural daylengths it is a practical part of the solution to produce inflorescences year around by delaying the flowering after the natural peak season when the prices are low. This approach is also easier to accomplish in the field than the use of artificial short days.

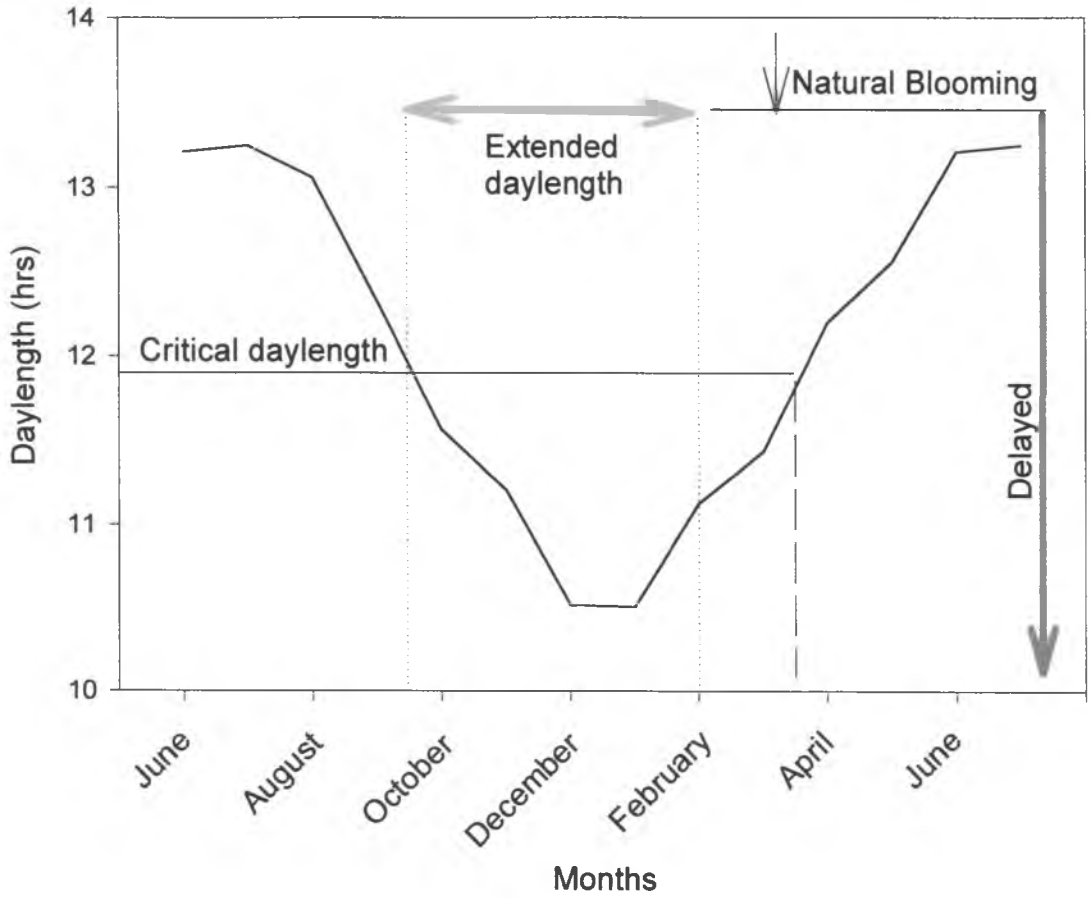


Fig. 5.3. Effects of extended daylength delaying the natural blooming season of *H. rostrata* in Hawaii. The critical daylength was close to the daylengths between March 1 to 15.

5.4.2. Supplemental daylength and night-break (Exp.5).

A total of 174 shoots were counted at the end of the experiment (August 1). The distribution of the shoots was 35.6 % under natural short-days, 32.8 % on night-break, and 31.6 % on supplemental-light (Table 5.1). Since 70 of the shoots had produced only 3 leaves by March 1, when the natural daylength was >11h 44', only 104 shoots with 4 or more leaves and therefore potentially inductive were used to analyze treatment effect. The number of usable shoots was similarly distributed for all treatments (Table 5.1)

Plants of *H. rostrata* cultivated under night-break and supplemental-day did not flower, but the ones under natural short-day did (Table 5.2). Both artificial daylength treatments inhibited flowering completely (0 %), whereas 28 % of the shoots under short-day flowered. The ANOVA showed significant ($P \leq 0.001$) treatments effects on flowering (appendix Table V.vi).

The first inflorescence for plants under natural SD appeared in the first week of May, and the inflorescence emergence period was from May to July. This period lies within the natural season (March to July) of the species under Hawaii conditions.

If the flowering was analyzed as percentage of flowered pots, it represented 66% of the pots, since 8 out of 12 pots flowered. The 2/3 flowering response as pots per treatment is similar to the results of the experiments 4.3.1 and 4.3.2. As with another SD heliconia, *H. wagneriana* (Criley and Sakai,1998) supplemental (extended) daylengths and night break lighting delayed or prevented flowering.

The mean number of inflorescences per flowered pot was 0.83 ± 0.72 (Figure 5.4). However, in two pots, 2 inflorescences per pot were observed. Figure 5.4 also shows the mean number of vegetative and dead shoots per pot. The number of vegetative shoots

Table 5.1. All and potentially-inductive shoots (> 3 leaves) of *H. rostrata* after 34 weeks of daylength treatments at the end of the experiment (August 1).

	Number and percentage of shoots			
Treatments	Natural Short-day	Night-break	Supplemental-light	TOTAL
All the shoots	62 (35.6 %)	57 (32.8 %)	55 (31.6 %)	174 (100 %)
> 3 leaves in first week of March	36 (34.6 %)	32 (30.8 %)	36 (34.6 %)	104 (100 %)

Table 5.2. Status of shoots (floral, vegetative and dead) in *H. rostrata* as total counts for the treatments natural SD, night break and supplemental light by August 1, 34 weeks after the onset of treatments.

	Number and percentage of shoots			
Treatment	Floral	Vegetative	Dead	Total
Natural Short-day	10 (28 %)a[1]	19 (53 %) b	7 (19 %)	36 (100 %)
Night-break	0 (0 %) b	24 (75 %) a	8 (25 %)	32 (100 %)
Supplemental-light	0 (0 %) b	31 (86 %) a	5 (14 %)	36 (100 %)
Significance[2]	***	**	ns	ns

[1]Mean separation of number shoots in each status within columns by Bonferroni multiple comparisons $P \leq 0.05$.

[2]Significance was determined by ANOVA of the counts per pot.

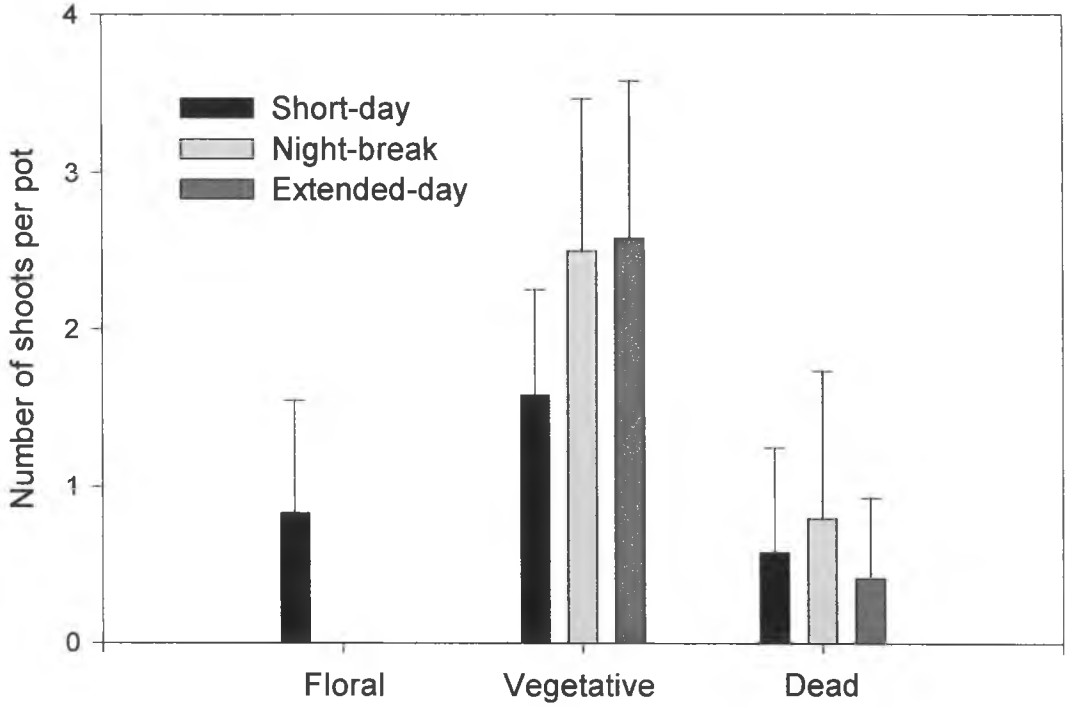


Fig. 5.4. Floral, vegetative and dead shoots of *H. rostrata* per pot under different daylength conditions.

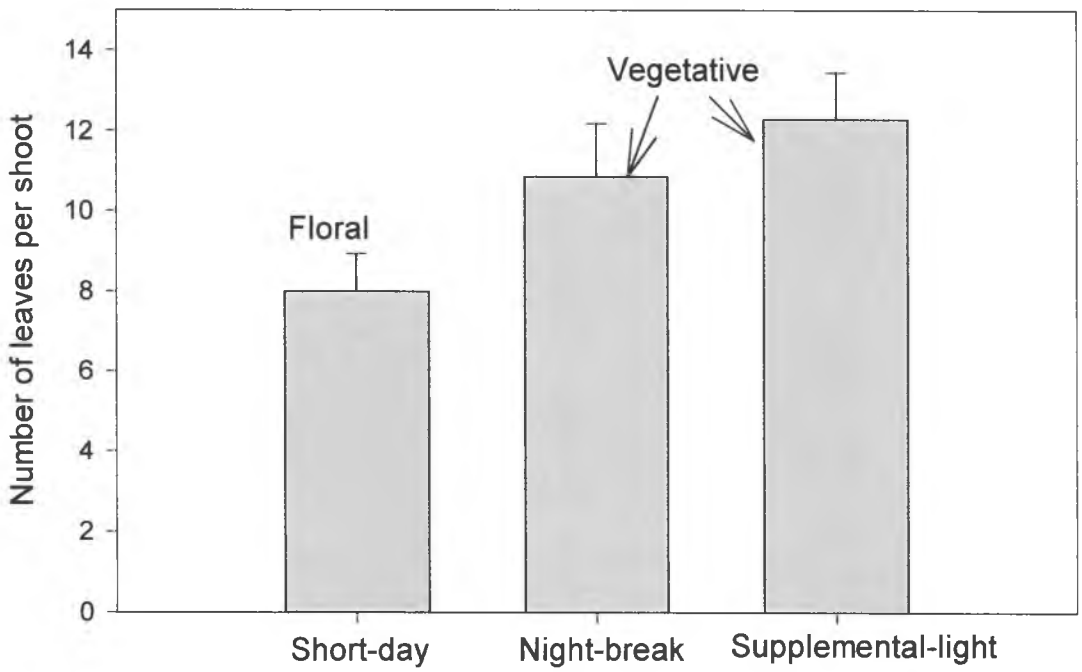


Fig. 5.5. Number of leaves on floral and vegetative shoots of *H. rostrata* per daylength.

was lower in plants under natural short days than in night-break and supplement-light. The dead shoot averages per pot were 0.58 ± 0.67, 0.8 ± 0.94 and 0.42 ± 0.0.51 respectively.

Vegetative shoots per treatment (Table 5.2) were affected by the daylength treatments (ANOVA, $P \leq 0.001$; appendix Table V.vii). The number of shoots in plants grown under natural short-day (19) was lower than in the ones under night-break (24) and supplemental-day (31). Both night-break and supplemental-day treatments were statistically equal. The number of dead shoots (Table 5.2) was not affected by the daylength treatments (appendix Table V.viii). Total shoot number (Table 5.2) was not affected by the treatments either (appendix Table V.ix).

The number of leaves in the flowered shoots ranged from 7 to 9. Figure 5.5 shows the mean and standard deviation of the number of leaves per shoot for all the treatments. The number of leaves in the shoots that were prevented from flowering by the extended day treatments was lower for shoots under night-break (ranged from 8 to 13) than under the supplemental-day treatment (ranged from 10 to 14).

Similar numbers of leaves subtending the inflorescence were reported in the previous experiment. Additional leaves (1.4) in shoots under supplemental light as compared to the ones in night-break may be explained as an effect of light in the continuation of the photosynthetic period or if the supplemental light was not sufficient to support photosynthesis could had a morphogenetic effect.

The natural SD did not induce flowering when coupled with short nights in the case of night-break, nor when supplemental daylength was supplied. Since no differences between treatments were observed for dead shoots, the difference in the status of the

shoots between the treatments was due to the shoots that were induced and able to flower (Table 5.2). As a consequence, it is possible to use either of the methods of extended daylength to control the vegetative growth, and thereby delay the flowering in *H. rostrata*.

It is well documented that light saturation to inhibit induction varies with species, conditions, and the time of exposure (Lumsden and Furuya, 1986; Thomas and Vince-Prue, 1997). If the critical daylength of *H. rostrata* was close to 11h 45' as was discussed in the previous experiment, it was possible that an extension shorter than the one used in this experiment but longer than 11h 45m could be enough to inhibit the induction. The night-break period could also be shortened.

From a practical standpoint, either night-break or extended-day could be used to inhibit flower induction in *H. rostrata*. From a commercial perspective, night-break would be more appealing than supplementary light, if it is applied in areas where night electricity tariffs are much cheaper.

5.4.3. Sequence Extended-natural long days (Exp.6)

A total of 72 shoots were counted in 16 pots from both daylength treatments on December 20. No statistical differences were found for the number of shoots between extended long day-natural daylength (37) and natural daylength (35) (Appendix Table V.x).

No inflorescences emerged in any of the treatments 28 weeks after the onset of the treatments. In induced plants, the emergence of the inflorescence generally occurred 21 to 24 weeks after induction (Chapters 4). Plant dissections showed an incipient reproductive stage in a few shoots. However, the induction of these shoots could not be attributed to

the treatments, since the stage of development was similar to the stages found in plants growing under natural short days by December (See Chapter 3). From this stage, the incipient inflorescence required between 8 to 10 weeks to emerge, scheduling the inflorescence emergence by the end of February or beginning of March. March is the beginning of natural season for *H. rostrata* in Hawaii. Plants under extended long-days-natural daylength (18 hours daylength for 8 weeks followed by natural decreasing long-days after July 13) behaved the same as the plants that were under natural daylengths.

No statistical differences were found between treatments for either the number of leaves per shoot at the onset of treatments and at the end of the experiment, or pseudostem height and location of the apex above the soil level (Appendix Tables V.xi to V. xiv). The mean number of leaves per shoot at the onset and at the end of the experiment (Figure 5.6) were similar for extended daylength (2.1 ± 0.91 and 7.2 ± 1.69) and for natural daylength (1.9 ±.1.26 and 7.5 ± 1.67). Pseudostem height (Figure 5.7) was higher under extended than under natural daylength (105 ± 23 cm and 90 ± 24 cm, respectively), however. The apex location, as height above the ground, was similarly located in both treatments (37 ± 23 cm and 28 ± 23 cm). The results also showed a direct relationship between apex location and pseudostem height.

Tallest shoots under extended daylength could be attributed to the effect of light quality. Treatments that reduce Pfr/Ptotal ratio in the range of 0 to 0.85 cause increased stem elongation (Vince-Prue, 1975; Thomas and Vince Prue, 1997). Since the light source used (incandescent or tungsten filament lamps) in this experiment is rich in far red, it could establish a low phytochrome fr:phytochrome ratio (Pfr/Ptotal).

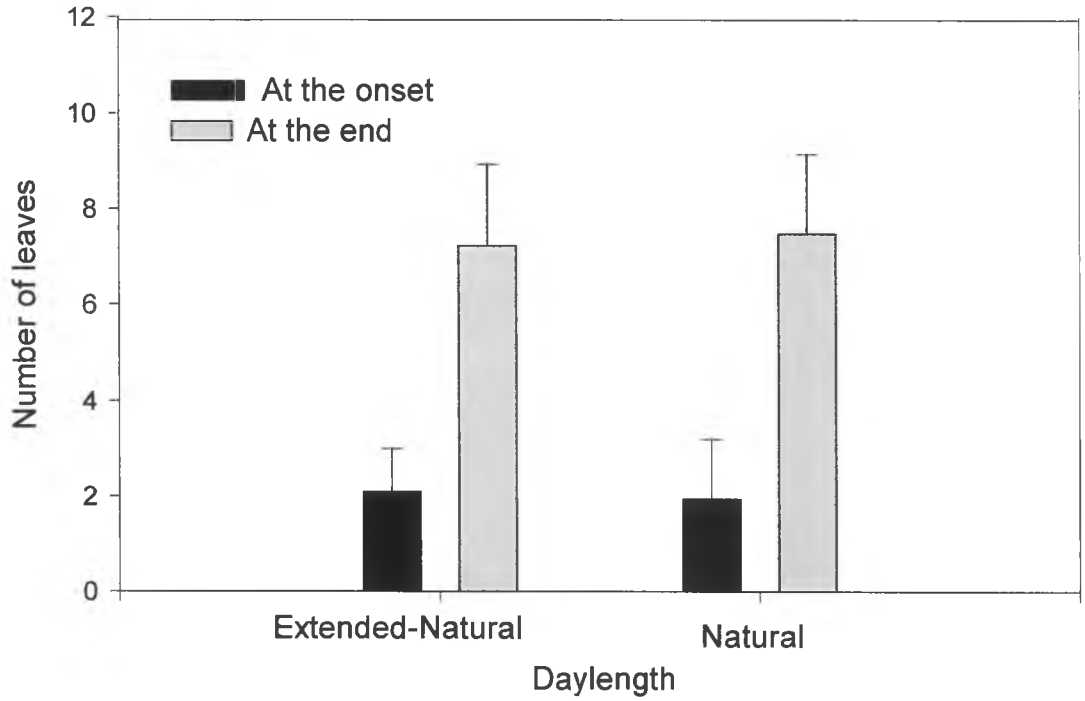


Fig. 5.6. Number of leaves at the onset and at the end of the experiment for shoots of *H. rostrata* under the sequence of extended long days-natural long days and only natural long days.

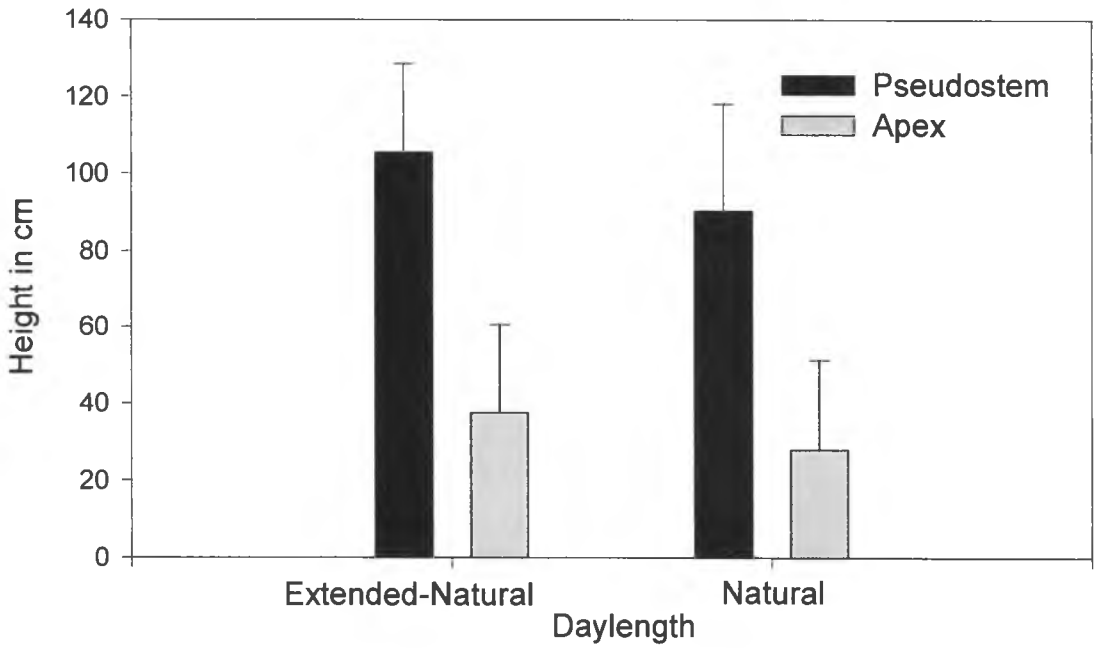


Fig. 5.7. Shoot height and apex location above soil level in *H. rostrata* under the sequence of extended long days-natural long days and only natural long days.

Since the simple change in daylength between two non-inductive photoperiods (dropping suddenly from 18 h to 13h 20m) was not effective to induce flowering in *H. rostrata*, then it seems clear that its flowering results from a critical SD daylength response not a change in daylength.

5.5. Conclusions

These results confirm the daylength response of *H. rostrata* found in previous chapters, and allowed us to qualify this species as a typical qualitative short-day plant. The critical daylength was between 11h 45m and 12h, hence light duration less than 11h 30m would induce flowering. Since flowering was inhibited by light, supplemental light or night-break can be used to extend the vegetative phase of growth in this species and to delay flowering if enough inductive short days remain to promote flower initiation and development following the flower inhibition. The approach of delaying flowering with light to extend the blooming period seems to be a practical alternative among the two strategies of daylength that can be used to manipulate flowering.

CHAPTER 6
SHOOT DENSITY, LEAF TEARING AND REMOVAL, AND DAYLENGTH ON THE FLOWERING OF *HELICONIA ROSTRATA*

6.1 Abstract

Two experiments were established to determine the effects of shoot density, leaf tearing and removal, and daylength on the flowering and shown the apex death.

One rhizome per pot was planted on July 97 and grown under long days (extended daylength 5 pm to 10 pm) from September 22 to February 1, when the four treatments were applied. All combinations of two shoot density treatments (one shoot and all shoots per generation) and two leaf tearing treatments (torn and untorn leaves) were applied by removing the shoots and tearing leaves in order to determine the effects on flowering and death. This procedure was continued as new shoots and expanded leaves developed in the following eight weeks. More inflorescences were developed on plants that had one shoot per generation than on plants with all shoots allowed to develop. At higher shoot density there were more dead shoots. There was no beneficial effect of leaf tearing on flowering. These results showed that the number of shoots in the clump reduced the flowering percentage of *H. rostrata* by increasing the number of shoots with dead apices. Since a certain amount of dead shoots still occurred in clumps with only one successor allowed per generation, other causes besides shoot density must play a role in shoot apex death.

Rhizomes were planted in 120 pots (8 L) in a factorial 3x2x2 design with 10 pots per treatment (one pot per replicate) in a second experiment. One third of the pots were planted with 2 rhizomes, while the remainder was planted with 1 rhizome. One half of the pots with 1 rhizome were allowed to develop all their shoots for 3 generations, while in

the remaining pots only 1 shoot per generation were allowed. In addition, one-half of the pots in all the treatments were subjected to selective leaf removal in order to determine the effects of shoot density and leaf removal on flowering and apex death. The plants were grown under LD until 4 leaves at the first generation were produced. Inductive SD was supplied for 8 weeks to all the plants from 5 pm to 8 am. After 8 weeks of SD, one-half of the plants were given extended LD (5:30 pm to 8:30 pm), while the other half continued under SD (conSD) until flowering. The results show that leaf removal did not affect the flowering *of Heliconia rostrata*. The highest percentage of flowering shoots (50 to 67 %) was observed under continuous SD from all generations of shoots at the end of the experiment; plants under SD-LD had 7 to 25 % flowering shoots. The second generation of shoots showed the highest flowering (80 % conSD and 28 % SD-LD), followed by the first (62 % conSD and 20 % SD-LD), and third (32 % conSD and 0 % SD-LD) generations. Non-flowering shoots of the first generation were aborted or had dead apices. Shoots of the third generation were still vegetative, since they had too few leaves to be induced. The results showed a differential response between floral and dead shoots with the generations. Independent of the treatments, the highest number of dead shoots occurred at the first generation. Even though shoot death was lower under conSD (8 to 20) than in SD-LD (33 to 52%) it is still a factor affecting flower production.

6.2 Introduction

Death of the shoot apex has been reported in different species of heliconias (Criley and Kawabata, 1986; Criley and Lekawatana, 1990; Criley and Lekawatana, 1995; Lekawatana, 1986; Lekawatana, 1995; Maciel, 1991). This phenomenon is an important factor affecting flower production in these species (Criley and Broschat, 1992).

In *H. stricta* 'Dwarf Jamaican', shoot apex death was associated with abortion of flower primordia during inflorescence development (Lekawatana, 1995). Lekawatana (1995) reported higher values of abortion on plants growing under higher nocturnal temperatures during the inflorescence development. However, it is unknown if temperature is the factor per se triggering the abortion of heliconias, since other environmental and endogenous factors have been also associated with flower abortion in many other plant species (Halevy, 1985; Abeles, et al, 1992).

Leaf tearing occurs commonly among members of the Musaceae, Heliconiaceae and Stelitziaceae, and this phenomenon was suggested to help plants with big lamina leaf area cope with the stress caused by high temperatures (Taylor and Sexton, 1972). These authors reported that sections of leaves less than 10 cm wide were not subject to critical heat stress (> 47.5 °C) in *Heliconia latispatha* and *Strelitzia nicolai*. In *H. latispatha*, leaf tearing resulted in 50% reduction of transpiration rate during the stressful time of the day, and the small size of leaf segments was favorable for net phothosynthesis during times of environmental stress. In bird of paradise (*Strelitzia reginae*) temperature was hypothesized initially as the factor inducing abortion (Criley and Kawabata, 1984: Kawabata et al., 1984). However, the high temperature hypothesis could not be substantiated, and later experiments supported nutritional competition among flowers as an explanation for flower abortion (Kawabata, 1989). Since this species has axial inflorescences (pleonanthic), the competition should occur between the large growing flower stalk and the differentiating inflorescence bud. A young developing flower bud is a major sink for assimilates under favorable growing conditions, if essential metabolites for growth are in ample supply, but it constitutes a weaker sink compared with the

vegetative apices under stress conditions with inadequate supply of assimilates (Halevy, 1987).

Plants grown in populations are morphologically and functionally different from plants grown as isolated individuals. Altered branching and assimilate allocation to reproductive structures and vegetative storage organs are among the responses to crowding (Ballaré et al., 1995). Since, in clumping plants different growth phases (vegetative and reproductive) are taking place simultaneously, the mixed allocation could be disadvantageous to reproductive organs (Bazzaz, 1997). In different grass and cereal species mutual exchange of metabolites occurs between parent and daughter tillers and between sister tillers (Marshall and Sgar, 1968; St-Pierre and Wright, 1972).

Heliconias are naturally clumping plants. The competition for available assimilates between the pseudostems in the clump could affect flower development and abortion. Observations of the previous experiments suggested a possible relation between the number of shoots in the clump and the flowering of *H. rostrata*; plants with a high number of shoots at the onset of SD showed the highest mortality of shoots (Chapter 4). In banana, a heliconia relative, selection of the sucker follower to synchronize and promote the production is an established horticultural practice. Lassoudiere (1980) reported that when all the suckers in the clump were permitted to grow, only two of them did flower, while the rest died. This author not only pointed out the interaction between the parent and daughter shoots but also between daughters.

Defoliation has been used to modify the flowering date in banana, and to some extent it has been associated with the reduction of the number of fruits per bunch. Shading can also be a major source of stress for plants of many crop species, determining

their reproductive allocation by early abortion of flowers and young fruits (Ballaré et al., 1995).

In the present chapter, two experiments are described. The objective in the first experiment is to analyze the effects of the competition among the shoots in the clump and the lower leaf temperature (potentially promoted by leaf tearing) on the number of floral or apex dead shoots. In the second experiment, the effects of density of shoots in the pot, leaf pruning, and the daylength after induction on flowering were studied.

6.3. Materials and Methods

6.3.1. Leaf tearing and shoot densities per clump (Exp. 7).

One heliconia rhizome per pot was planted, in July 1997, into 24 plastic pots (18 cm diameter x 15 cm depth) containing a mixture of peat, perlite, composted redwood, and volcanic cinders at 1:1:1:1 (by volume) proportions. The potting mixture was amended with dolomite, Micromax^{TM} (minor elements) and treble superphosphate at rates of 6.0, 1.0 and 0.6 kg/m^3, respectively. The plants were irrigated twice daily, with nutrient solution (200 N- 0 P- 233 K ppm) and maintained at the Magoon Horticulture facility saranhouse (30 % shade) until September.

On September 22, plants were transferred to Pope laboratory glasshouse and provided an extended daylength from 5 pm to 10 pm until February 1. The source of light was incandescent lamps of 100 watt (3.8 W.m^{-2}) placed 1.6 m above the pots. During this experimental phase, plants were watered by overhead sprinklers twice a day and fertigated, twice a week, with 1000ml/pot of nutrient solution of 500 ppm N-P_2O_5-K_2O at the ratio of 20-20-20.

On February 1, four treatments in a randomized design with 6 single-pot replications were set up. The treatments were clumps where only one shoot per generation (parental, daughter and grandson shoot) was allow to grow, and clumps in which all shoots were allow to grow. For single shoots per generation, the most vigorous shoot from each generation was the one allowed to grow. Each of the two shoot density treatments was divided into two subgroups; a first subgroup with intact leaves and a second subgroup with lacerated or torn leaves. In shoots with 3 or more unfurled leaves, all leaf laminas were torn by hand at 5 cm intervals following the parallel veins, simulating natural tearing. Both leaf tearing and shoot removal were applied to newly expanded leaves and shoots during the eight weeks following the end of daylength extension. The period of tearing was based on the assumption that induced plants will continue the development and appearance of leaves already formed at the apex in the weeks following the onset of induction (experiment 4.3.1, Figure 4.2).

The newly expanded leaves were labeled every week. The number of shoots per pot and their status (floral, vegetative and dead) and number of leaves per shoot were determined by dissection of the pseudostem at the end of the experiment in August 1998.

6.3.2. Leaf removal, shoot density per pot and daylength (Exp. 8).

Separate rhizome pieces of *H. rostrata* were potted in 120 pots (8 L) in August 1998. The potting mixture and amendments were the same as described in the previous experiment (5.3.1). One third of the pots were planted with 2 rhizomes, while the remainder was planted with 1 rhizome. One half of the pots with 1 rhizome were allowed to develop all their shoots for 3 generations, while in the remaining pots only 1 shoot per generation was allowed to grow. Plants were placed at Pope Laboratory glasshouse.

Supplemental light was supplied from 5:30 pm to 8:30 pm from October 1 to November 15. The source of light was incandescent floodlamps (19 $W.m^{-2}$). At the end of supplemental light period, the shoots of the first generation (parent) had reached four leaves, and most of the shoots of the second generation (daughter) had one leaf. However, in a few of the most advanced shoots six and three leaves were counted for the respective generations. Plants were irrigated twice daily by overhead sprinklers, and fertigated, once a week with 1000ml/pot of nutrient solution of 500 ppm $N\text{-}P_2O_5\text{-}K_2O$ at the ratio of 20-20-20. One tablespoon of $N\text{-}P_2O_5\text{-}K_2O$ at the ratio of 19-6-12 from a slow release fertilizer (Osmocote®) was applied to each pot on October 3 and March 1. The maximun air temperature range during this experiment was 26 to 42 °C, with a mean of 33 °C, and the minimum temperature ranged from 19 to 26 °C with a mean of 22 °C.

Inductive SD treatments started on November 15. Plants were provided with 9 hours of natural daylight by covering them daily from 5:00 pm to 8:00 am with black plastic curtain. Eight weeks after the onset of SD, one half of the plants (60 pots; 20 pots from each shoot density treatment) were provided LD by extended daylengths from 5:30 pm to 8:30 pm (SD-LD) until March 1 (14 weeks from the onset of inductive SD). The light source was the same as in the supplemental light phase. The remaining 60 pots continued under 9 hours short days (conSD) until flowering. Half of each daylength treatment group of plants (10 pots per each treatment) was subjected to selective leaf removal or pruning. The most recent expanded leaf lamina was completely removed in shoots with more than four leaves at the onset of SD. From the start of SD until 14 weeks later, each new completely expanded leaf was removed weekly. The removal of shoots in the one shoot per generation treatments also continued for 14 weeks after the onset of SD.

A factorial design with 3 shoot density, 2 daylength, and 2 leaf removal levels was used. Each of the twelve treatments consisted of 10 single pot replicates. The plants were placed on four benches. Two of the benches held the plants of SD-LD randomly distributed, while on another two benches were placed the conSD plants.

The leaves were tagged at the onset of the treatments and weekly as each new leaf expanded. The number of shoots per pot, their status (floral, vegetative and dead) per generation and the number of leaves and height of the flowered shoots were determined at the end of the experiment in October 1999. As described for the previous experiments, the status of vegetative and dead shoots was determined by dissection of the pseudostem.

6.3.3. Statistics

Since the number of shoots was also a treatment, the analysis after effect was performed on the proportion (in percentages). The total number of shoots per pot was considered as 100 %, and the shoots by status (floral, vegetative and dead) as the respective proportion. These values were transformed to obtain normal distribution of residuals, and equal variances among group (Little and Hills, 1978). Data from all the experiments were subjected to ANOVA, mean separation by Bonferroni multiple comparisons, and regression when required using the statistical program SYSTAT 7.0 for windows (SYSTAT, 1997).

6.4. Results and Discussion

6.4.1. Leaf tearing and shoot density by clump (Exp. 7)

A total of 130 shoots were counted at the end of this experiment in August 1998. An average of three generations of shoots developed during the 12 month period of the experiment. However, four generations were observed in some of the pots. When four

generations were observed, only shoots from the first to the third generation had sufficient time to develop and flower. The emergence of inflorescences started on the third week of July, 24 weeks after the start of SD.

The distribution of total shoots was 2.5 times higher for the treatments where all the shoots were grown per generation than when only one shoot per generation was allowed to grow (Table 6.1).

A higher proportion of floral shoots (61 and 58 %) developed when plants were limited to one shoot per generation than when all shoots were allowed to developed (11 and 4 %) (Table 6.1). Number of shoots per generation (one or more) affected significantly ($P \leq 0.001$) the floral shoot percentage by pot. Differences for vegetative ($P \leq 0.01$) and dead ($P \leq 0.001$) apex shoots were also observed between plants with one and all shoots per generation in the pot (Appendices Table VI.ii to iii).

No differences were observed for leaf tearing and the interaction of both treatments (Appendix Table VI.i). Plants with one shoot per generation had lower percentages of vegetative (21 to 22 %) and dead apex shoot (17 to 21 %) than did plants with all shoots allowed to develop (36 to 24 % and 53 to 72 %, respectively).

These results show that the number of shoots allowed to grown per generation are related with the number of inflorescences and dead shoots. Results where floral and dead shoots are inversely related have been already discussed in the previous chapters. These results also shows that the vegetative shoots tend to be less variable among the treatments than the flowered and dead ones, since they represent mainly the last generation of individuals in development.

6.1. Distribution of the total number and percentage of shoots of *H. rostrata* by status (floral, vegetative and dead) and treatments (density of shoots and leaf tearing).

	Status			
Treatments	Floral	Vegetative	Dead	Total
One shoot/generation & torn leaves	11 (61 %)	4 (22 %)	3 (17 %)	18 (100 %)
One shoot/generation & intact leaves	11 (58 %)	4 (21 %)	4 (21 %)	19 (100 %)
All shoots/generation & torn leaves	5 (11 %)	17 (36 %)	25 (53 %)	47 (100 %)
All shoots/generation & intact leaves	2 (4 %)	11 (24 %)	33 (72 %)	46 (100 %)
			Total	130
Analysis of Variance significance for percentage of shoots per pot				
Density	***	***	***	
Tearing	NS	NS	NS	
Density x Tearing	NS	NS	NS	

NS, *** Nonsignificant or significant at $P \leq 0.001$, respectively.

Even though the number of dead shoots was lower in clumps with only one shoot by generation than when all shoots were allowed to develop, a considerable percentage of death still occurring at the apex of *H. rostrata*. The percentage of dead shoots was higher in shoots with torn leaves versus intact leaves (21%-torn versus 17 %-intact in clumps with one shoot generation and 53 %-torn versus 72 %-intact in the clumps with all the shoots per generation). Values of dead shoots close to 20 % were observed in experiment 5.4.2 for plants under short days, night break and supplemental light (19, 25 and 14 %, respectively). Thus this value could represent natural shoot death rates inherent to the species. However, natural or not, there impact still be a cause for shoot death. In *H. stricta,* under environmental controlled conditions no shoot death was observed when the plants grown under continuous SD versus 20 % shoot death under continuous LD (Lekawatana, 1995).

The floral shoots produced an average of 10.4 $\pm$ 2.1 and 9.2 $\pm$ 1.0 leaves for plants from one shoot per generation and all shoots per generation respectively, while the numbers of leaves at the onset of SD for these shoots were 5.8 $\pm$ 2.3 and 4.7 $\pm$ 1.3 (Figure 6.1). The variation in number of leaves between both treatments resulted from the few floral shoot at high density and these shoots had fewer leaves.

The number of shoots per pot at the end of the experiment ranged from 3 $\pm$ 0.89 to 7.8 $\pm$ 2.14. Figure 6.2 shown the number of shoots per pot or clump by status (floral, vegetative and dead) and treatments (shoot density and leaf condition). Pots with lower density of shoots (3 to 3.2 shoots) had the highest number of inflorescences (1.8) per pot and lowest number of dead shoots (0.5 to 0.7). At the higher density, the floral shoot number varied from 0.33 to 0.83, while the dead ones ranged from 4.2 and 5.5. The

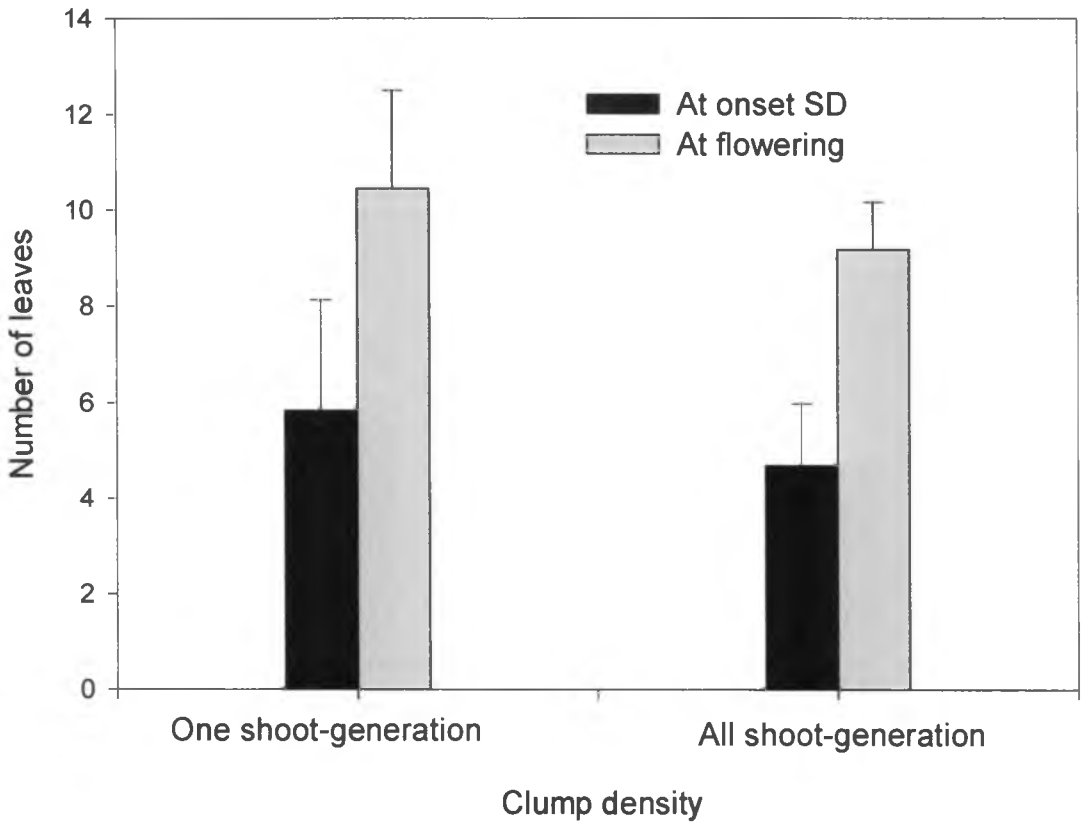


Fig. 6.1. Number of leaves per floral shoot of *H. rostrata* by shoot density at the onset of SD and at flowering. Vertical bars are one standard deviation.

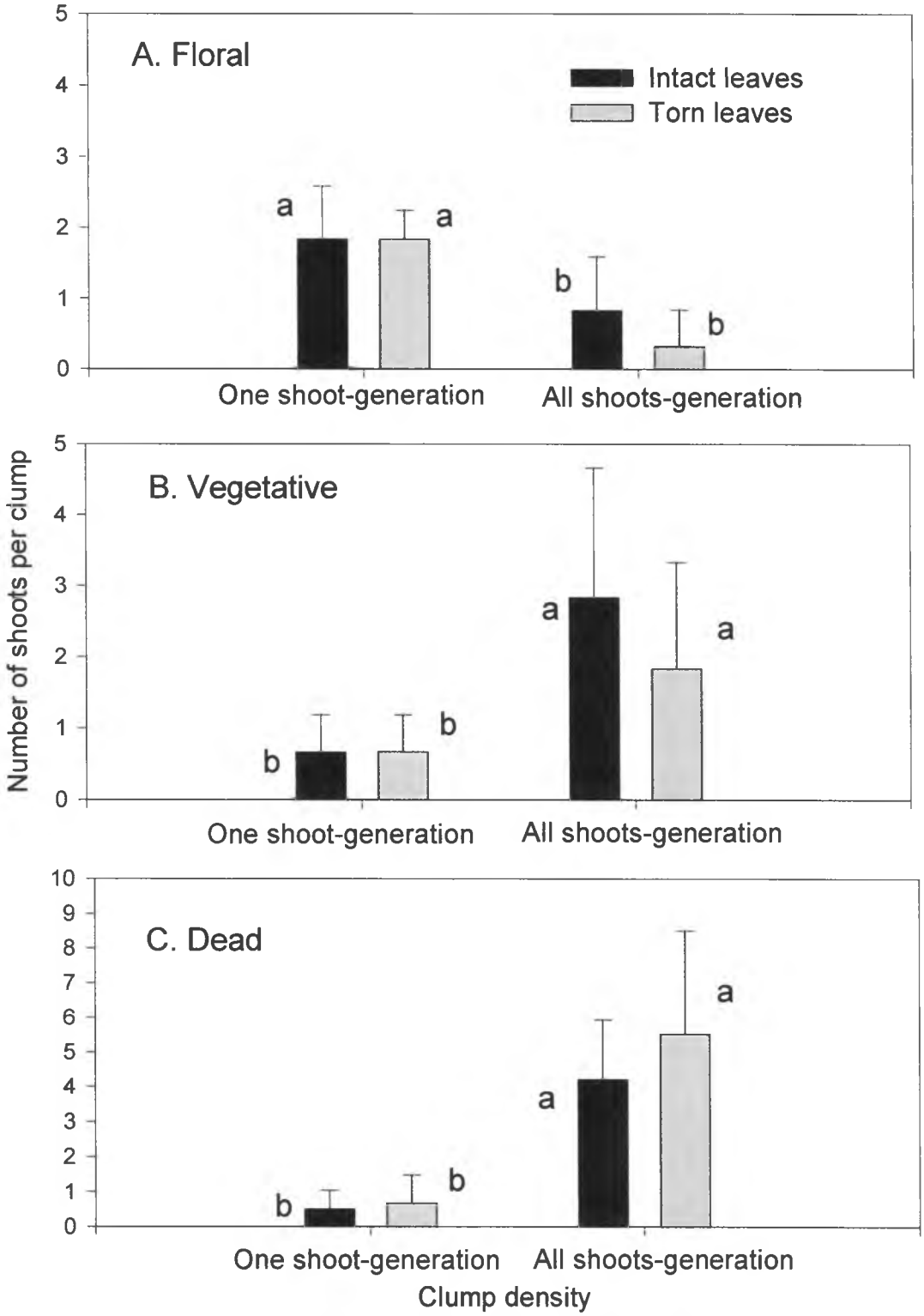


Fig. 6.2 Number of floral (A), vegetative (B), and dead (C) shoots per pot in *H. rostrata* by shoot density and leaf condition. Mean separation (a, b) within leaf tearing by Bonferroni multiple comparisons.

means were statistically different (using Bonferroni multiple comparisons test, $P \leq 0.001$) within each of shoots density treatment.

Neither negative or positive effects were found following leaf tearing, on floral and dead shoots under the experimental conditions. Thus, the hypothesis that shoots with torn leaves could reduce the death of shoots was not supported for *H. rostrata*. This could be because temperature was not the factor promoting the death of the shoots. However, the hypothetical reduction of temperature of leaves caused by tearing as reported under natural habitat for *H. latispatha* by Taylor and Sexton (1972), could also be not enough to allow the plant cope with heat stress in the glasshouse where the air circulation is lower than in open spaces. In addition, the maximum air temperature in the glasshouse was not over 42 °C (below the critical 47.5 °C reported by Taylor and Sexton (1972) for banana, leaves), and the plants were not under water deficit that could accentuate temperature stress.

Clumps with one shoot per generation had fewer dead shoots than did plants with many shoots. This could be a consequence of the effects of crowding in the allocation to reproductive structures (Harper, 1977). A better survival of shoots in heliconia should be expected when fewer shoots are present in the clump to compete for a limited supply of carbohydrates, minerals, water, light, etc., than when multiplestems are present in the clump, such was reported for banana (Lassoudierre, 1980). On the other hand, in barley, primordium formation stopped abruptly in plants at high density. After the death of the terminal primordia was observed the elongation of the rachis internodes. The death of the heliconia apex may be related with the apex has no vascular tissue and the movement of nutrients take place by diffusion from a disc of vascular tissue formed at the nodes (Kirby

and Faris, 1970). However, there is still the possibility that the death of shoots was accentuated as a consequence the simultaneous induction of more shoots.

The results of this experiment support the hypothesis that the number of individuals in the clump affected the flowering of *H. rostrata* by promoting the number of dead shoots.

6.4.2. Leaf removal, shoot density and daylength (Exp. 8)

A total of 692 shoots were counted from three generations of shoots over a period of 14 months at the end of the experiment. These shoots corresponded to: 332 in pots with one rhizome in which all the shoots were allowed to grow; 120 shoots in pots with one rhizome and one shoot per generation; 240 shoots in pots with two rhizomes and one shoot per generation. After 14 months the percentage distribution of floral, vegetative and dead shoots was approximately equal as shown in Figure 6.3.

The average of shoots per pot was 8.3, 3 and 6 for pots with one rhizome and all the shoots per generation, one rhizome and one shoot per generation, and two rhizomes and one shoot per generation, respectively; it represented a relation of 2.8:1:2 shoots per generation per pot. Figure 6.4 shows the number of shoots by status (floral, vegetative and dead) for different densities of shoots and daylength treatments per pot.

The emergence of the inflorescences started 24 weeks after the onset of SD (April 26) in both continuous SD and the SD-LD conditions. For plants grown under SD-LD, flowering occurred from the week 24 until the week 32 (9 week period), while in plants under conSD it was prolonged until early September (20 weeks period).

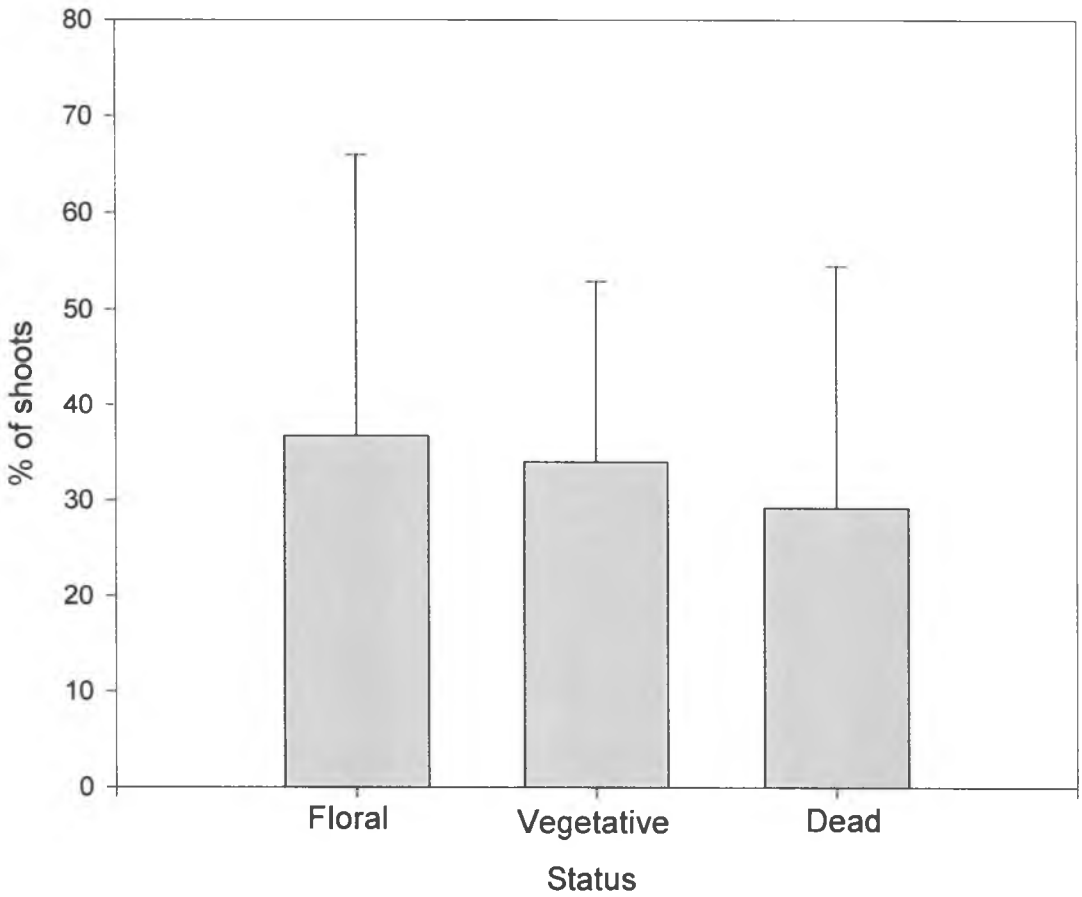


Fig. 6.3. Distribution of shoots as percentage by status (floral, vegetative and dead) in *H. rostrata*. Vertical bars are standard deviations.

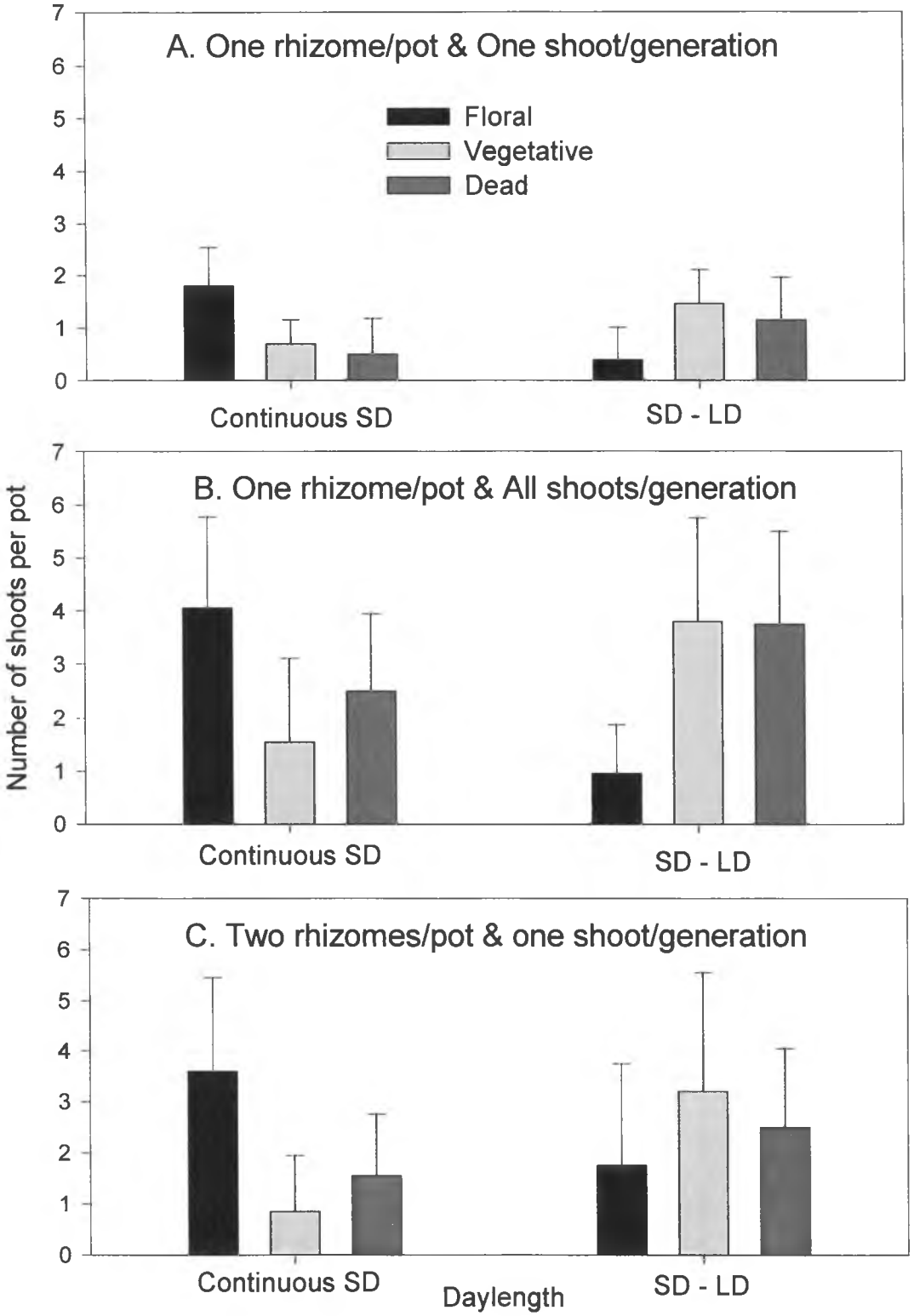


Fig. 6.4. Shoots per pot in *H. rostrata* by shoots density (A, B, C), status (floral, vegetative, dead) and daylength. Vertical bars are standard deviations.

The numeric distribution of all shoots by treatments (shoot density, daylength and leaf pruning) and their respective proportion as percentage of all the shoots per pot (100%) are shown in Table 6.2. The number and percentage of floral shoots were greatest under continuous SD across all shoot densities and leaf removal treatments. On the other hand, dead shoot numbers were lowest under continuous SD. Vegetative shoot numbers varied less among the treatments than did the floral and dead shoots. However, there were more vegetative shoots under SD-LD than under SD treatments.

The highest percentages (50 to 67 %) of floral shoots by shoot density per pot were observed in the plants under continuous SD (Table 6.2). The lowest percentages (7 to 25 %) of floral shoots occurred under SD-LD. Lower percentages of vegetative shoots (from 23 to 32 %) were observed under continuous SD than under SD-LD (33 to 54 %). The lowest percentages of dead shoots, from 8 to 20 %, were observed under continuous SD, while the highest percentages varied from 33 to 52 % under SD-LD.

The percentages of floral, vegetative and dead shoots of the total shoots per pot (100%) were separately analyzed for the different treatments by ANOVA. Statistical differences ($P \leq 0.001$) were detected among floral, vegetative and dead shoots for the daylength treatment (Table 6.2; Appendix Tables VI.iv to VI.vi). No differences were observed for shoot densities and leaf removal treatments.

Because more shoots were under induction during continuous SD more floral shoots were expected. But, since Lekawatana (1995) did not observe apex death in *H. stricta* under continuous SD, daylength was included to be tested. The results in this experiment shows that even when the apex dead was lower under continuous SD (8 to 20 %) when compared with SD-LD (33 to 52 %) it was not eliminated in this species.

Table 6.2. Distribution of the total number and percentage of shoots of *H. rostrata* by status (floral, vegetative and dead) and treatments (daylength, density and leaf pruning).

Treatments	Status			
	Floral	Vegetative	Dead	Total
One rhizome/pot & One shoot/generation				
Continuous SD & unpruned leaves	18 (60 %)	7 (23 %)	5 (17 %)	30 (100 %)
Continuous SD & pruned leaves	18 (60 %)	7 (23 %)	5 (17 %)	30 (100 %)
SD - LD & unpruned leaves	4 (13 %)	16 (54 %)	10 (33 %)	30 (100 %)
SD - LD & pruned leaves	4 (13 %)	13 (43 %)	13 (44 %)	30 (100 %)

One rhizome/pot & All shoots/generation				120
Continuous SD & unpruned leaves	38 (50 %)	24 (32 %)	14 (18 %)	76 (100 %)
Continuous SD & pruned leaves	43 (50 %)	26 (30 %)	17 (20 %)	86 (100 %)
SD - LD & unpruned leaves	6 (7 %)	37 (41 %)	47 (52 %)	90 (100 %)
SD - LD & pruned leaves	13 (16 %)	38 (48 %)	29 (36 %)	80 (100 %)

Two rhizomes/pot & One shoot/generation				332
Continuous SD & unpruned leaves	40 (67 %)	15 (25 %)	5 (8 %)	60 (100 %)
Continuous SD & pruned leaves	32 (53 %)	16 (27 %)	12 (20 %)	60 (100 %)
SD - LD & unpruned leaves	15 (25 %)	21 (35 %)	24 (40 %)	60 (100 %)
SD - LD & pruned leaves	9 (15 %)	20 (33 %)	31 (52 %)	60 (100 %)

				240
Analysis of Variance significance for percentage of shoots per pot				
Density	NS	NS	NS	
Daylength	***	***	***	
Leaf pruning	NS	NS	NS	
Density x Daylength	NS	NS	NS	
Density x Leaf pruning	NS	NS	NS	
Density x Daylength x Leaf pruning	NS	NS	NS	

NS, *** Nonsignificant or significant at $P \leq 0.001$, respectively.

The distribution of shoots per generation was also studied. ANOVA detected similar significant differences for daylength among the floral, vegetative and dead shoots of each of the three generations as found for the previous analysis pooling all shoots together (Appendices Tables VI.vii to VI.xv). But for the third generation it also detected differences among dead shoots for the shoot density treatments ($P \leq 0.001$) and the interaction daylength x shoot density ($P \leq 0.05$). Figure 6.5 shows the distribution as percentages of the floral (A), vegetative (B) and dead (C) shoots by daylength and generation. The highest percentages of floral shoots occurred in the second generation of shoots in both daylength conditions (80 and 28 % respectively). The lowest percentage of floral shoots occurred in the third generation (32 and 0 %). In contrast, the highest percentages of vegetative shoots occurred in the third generation; 66 and 79 % for continuous SD and SD-LD, respectively. The highest percentages of dead shoots were observed for the first generation of shoots; 37 and 73 % for continuous and SD-LD, respectively.

Almost all of the non-flowering shoots of the first generation were dead, with only 2 % remaining vegetative (Figure 6.5 B). The dead shoots at the third generation were mainly the ones that were not induced because of their young stage (less than 3 leaves) for shoots under continuous SD, and/or the ones that were not under inductive light condition, such as the ones that reached the minimal number of leaves after the 8 weeks period of SD in the treatment SD-LD (see the zero value of floral shoots in the third generation in Figure 6.5 A and 79 % of vegetative shoots in Figure 6.5 B). However, twenty one percent of shoots under SD-LD were dead (Figure 6.5 C).

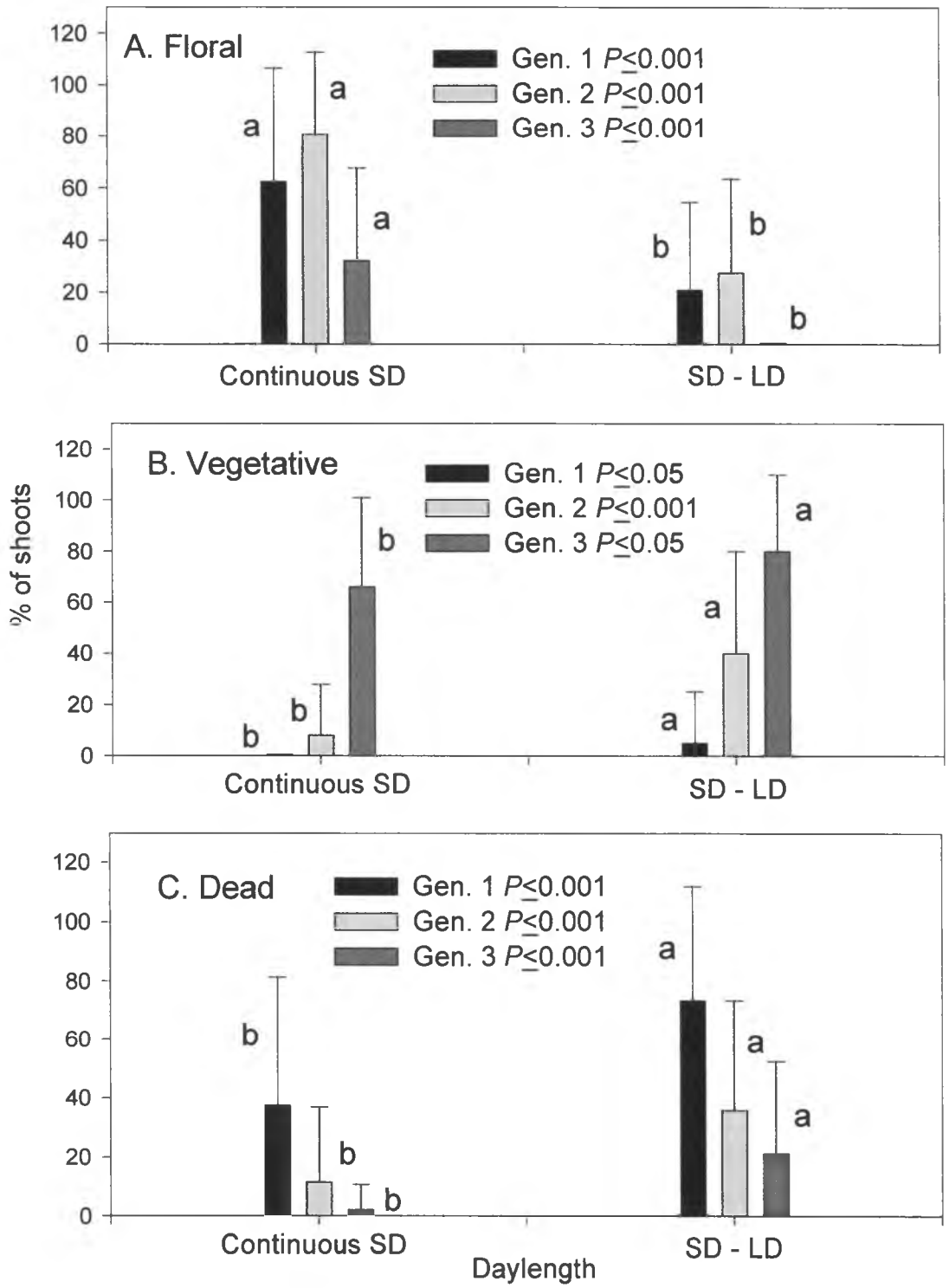


Fig. 6.5. Distribution of shoots as percentage by pot in *H. rostrata* by status (A, B, C), generation and daylength. Vertical bars are standard deviations, with mean separation (a,b) for daylength treatment within generation and status of shoot by Bonferroni multiple comparisions.

The 8 % dead shoot proportion observed for one of the treatments under conSD in this experiment could be attributed to the natural death threshold suggested in the previous experiment. However, in *H. stricta* under continuous SD Lekawatana (1995) reported 0 % of death.

On the other hand, a portion of the dead shoots (from 20% to 52 %) observed under SD-LD was a consequence of shoots that were subjected to inductive conditions for a period of time insufficient to complete the induction process. An apex insufficiently induced could fail to develop flowers and/or leaves and thus reach a dead end. Incomplete induction could trigger the death of the shoots.

Figure 6.6 shows the number of leaves (A) and pseudostem height (B) in floral shoots by generation and shoot density. The number of leaves decreased with the generation in all the densities, while the height of the pseudostem increased. The shoots with fewer leaves were the ones from plants where all the shoots were allowed to grow, while the smaller in size plants were found in pots with two rhizomes and one shoot per generation. Since the number of leaves tend to decrease for secondary and tertiary shoots in the same generation, the lowest number of leaves for shoots in pots with one rhizome and all the shoots could be a consequence of that decreasing in number of leaves.

Shoot generation has also been reported similarly affecting the final leaf number and shoot height of floral shoots in *H. bihai* and *H. rostrata* (Maciel, 1991; Maciel and Rojas 1994); and affecting leaf number in *H.* 'Golden Torch' (Catley and Brooking, 1996). Lekawatana (1986) suggested that the increased height of shoots with generation number in *H. stricta* may have been the effect of the increased food reserves or a consequence of crowding which caused the plants to stretch. However, the tallest shoots

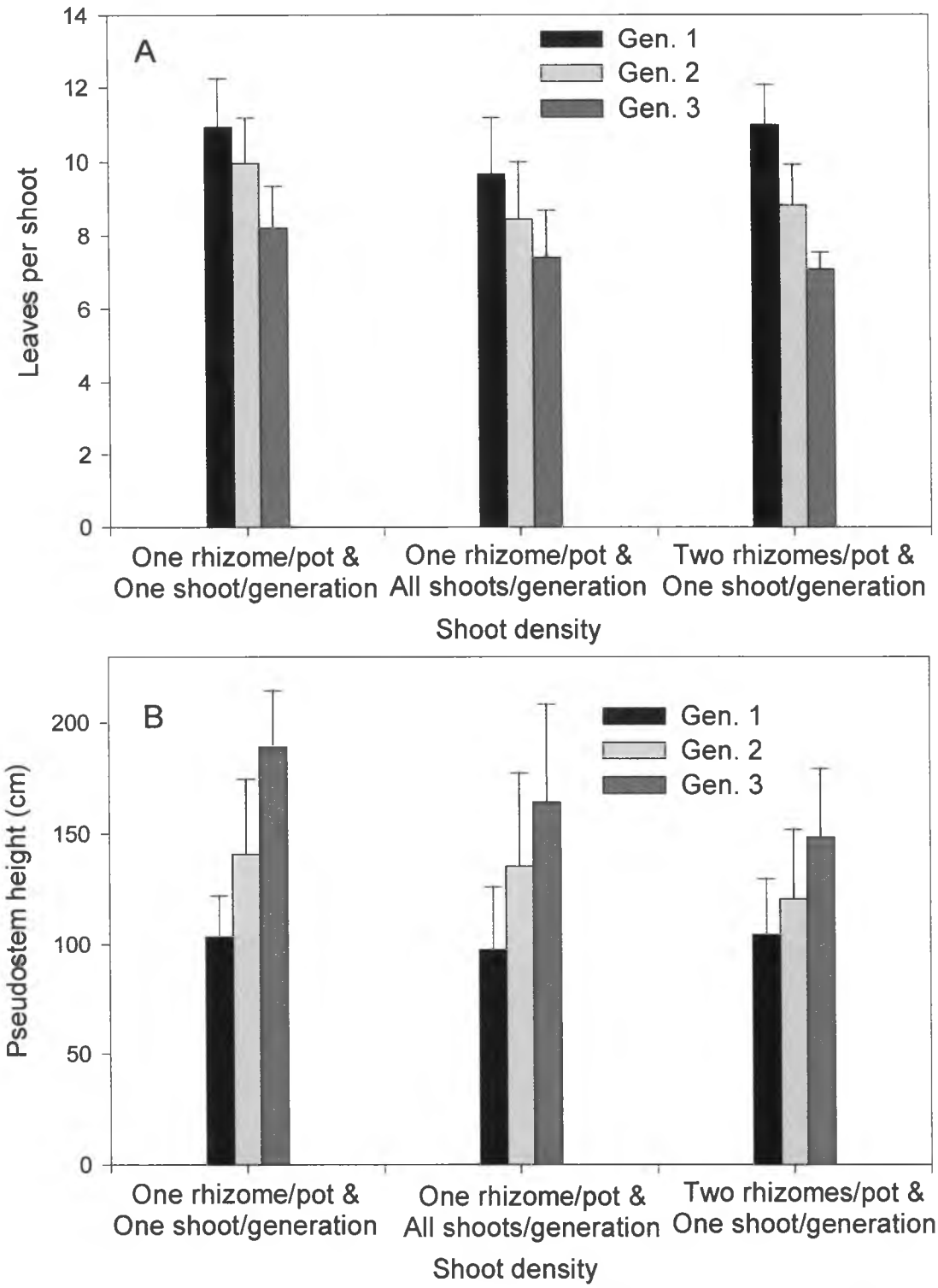


Fig. 6.6. Number of leaves (A) and pseudostem height (B) of floral shoots in *H. rostrata* by generation and shoot density. Vertical bars are standard deviations.

were not the ones at the higher density (Figura 6.6B), therefore other factors beyond shading by crowding should be involved.

The results of this experiment support the explanation of increased reserves with the succeeding generations of shoots. The decreased in number of leaves with generation number also supports this hypothesis. Plants from the previous experiment (Exp. 7) had more leaves (average of 5.3 per shoot, Fig.6.1) at the beginning of SD induction than in this one (4 leaves). It is important to consider that floral shoots of the third generation correspond at treatments under conSD. This could explain why more shoots under SD-LD flowered in that experiment than in this one. Since in this experiment, the shoots were younger (more "juvenile") and probably with less accumulated reserves they may have been only marginally sensitive to be induced. In many monocarpic perennial plants a minimum size is required for plants to be competent to be induced (Reekie, 1997). In plants such as rice, seedling vigor is considered a factor affecting the degree of sensitivity to photoperiod (Yin et al., 1997).

ANOVA detected a significant difference for vegetative and dead shoots of the third generation ($P \leq 0.05$) but not for first or second between plants with one shoot per generation (group 1) and all shoots (group 2) (Figure 6.7). No differences for floral shoots in any generation were detected (Appendix Tables VI.xvi to VI.xxiv). This shows that some of the effects of shoot density were not detected as consequence of the variance among the treatments when all the treatments were analyzed together.

When the competition between clumps (Figure 6.8) was analyzed by comparing between one rhizome per pot and all the shoots (group 2) and two rhizomes per pot (group 3) the differences were found in the second generation for floral and vegetative

shoots (Appendix Tables VI.xxv to VI.xxxiii). The differences for floral shoots between the groups 2 and 3 and dead shoots between group 1 and 2 were both associated with vegetative shoots.

Plants with more competing shoots (in the clump group 2) showed high shoot death for the third generation (Figure 6.7) while shoot death was only slightly lower on pots with 2 rhizomes (group 3) (Figure 6.8). The percentages of floral shoots were higher for second generation shoots for the treatments one and two rhizome per pot. Similar values were observed for the first and second generation of floral shoots for plants where all the shoots were allowed to grow in the clump.

Shoots of the second generation might be the ones with more reserves accumulated and in a stage of growth to be more competitive than the shoots of the first generation. Even though, there is no information about the translocation of substances, minerals and water between shoots in the clump of heliconia, however it occurs in banana (Stover and Simmonds (1987). For example, it is known that the root system of banana shoots decrease in efficiency with age and the older shoots in the clump depend on the new shoots to continue their growth. If more plants had already reached their competent stage and are simultaneously induced to flower, more competition among higher number of induced shoots could occur.

Even though the discrepances between the results of both experiments could be discussed from different approaches, there is a good possibilty that the stage of growth of plants at the moment of the induction was one of the factors that marked these differences. On the other hand, since continuous SD following the induction period

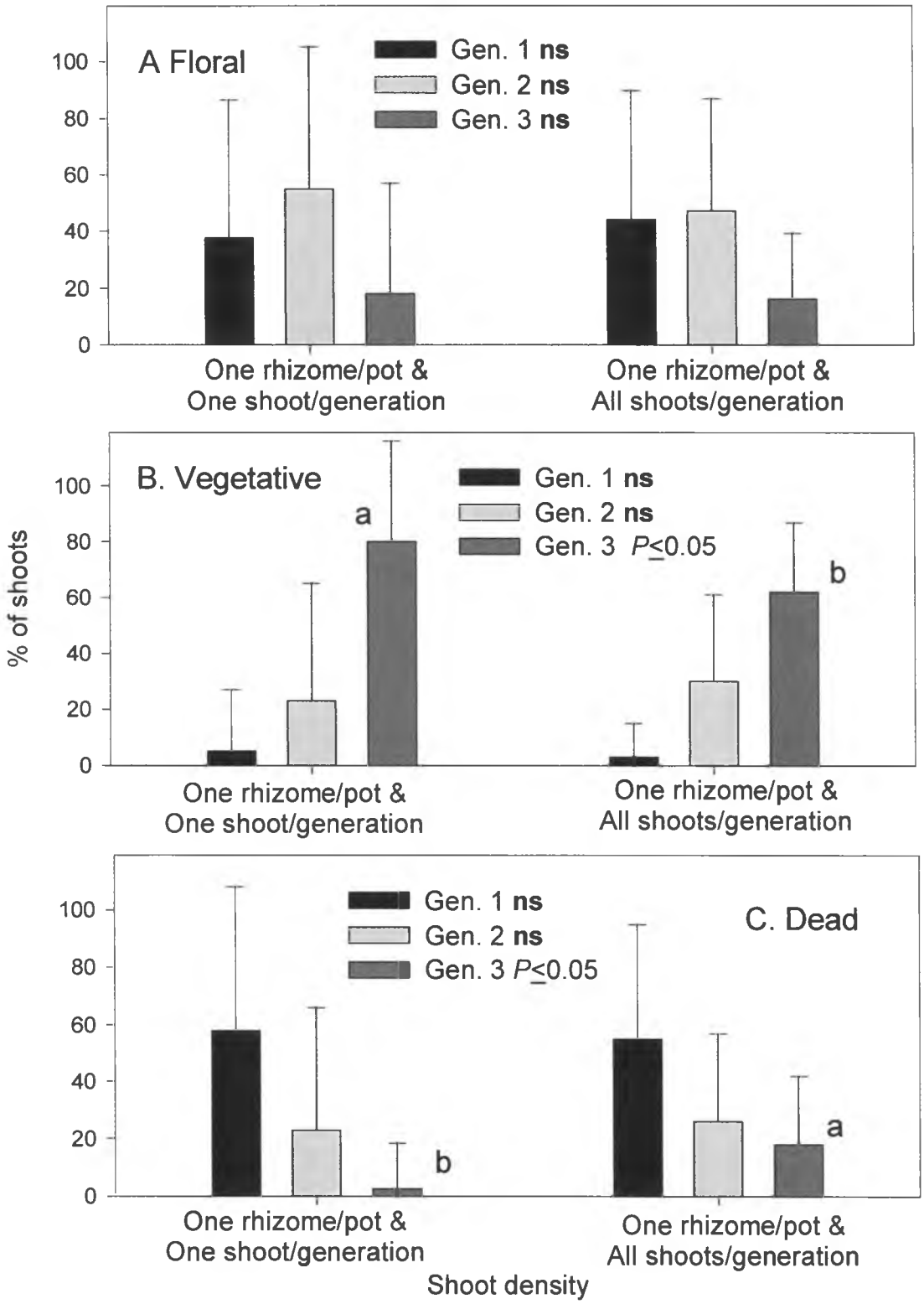


Fig.6.7. Shoots as percentage in *H. rostrata* by status (A, B, C), generation and shoot density. Vertical bars are standard deviations,with mean separation (a,b) within a generation by density using Bonferroni multiple comparisons.

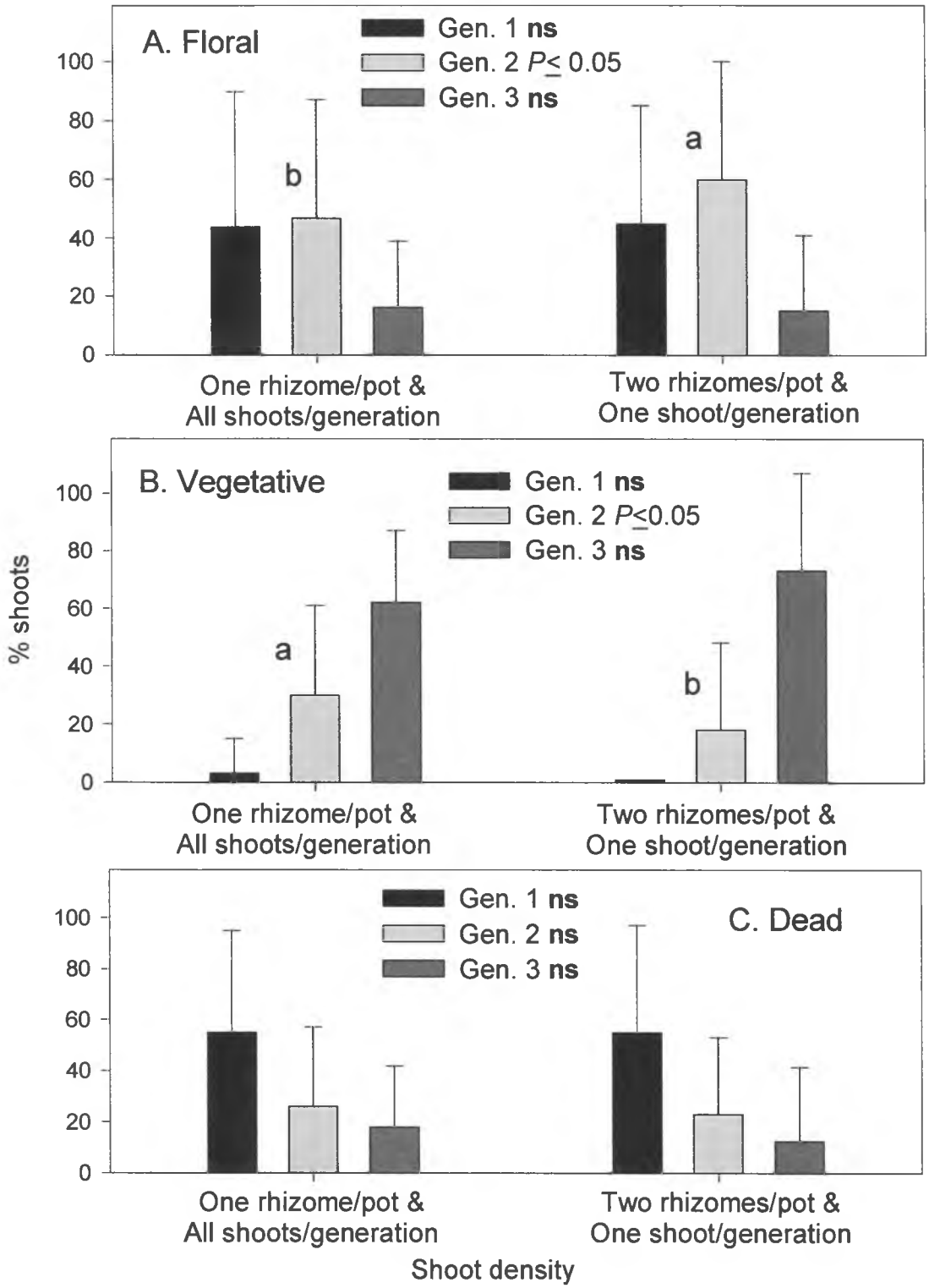


Fig. 6.8. Shoots as percentage in *H. rostrata* by status (A, B, C), generation and shoot density. Vertical bars are standard deviations, with mean separation (a,b) within a generation by density using Bonferroni multiple comparisons.

reduced the death, and this equatorial species is induced and differentiates their flowers under natural short days (already differentiated by January in Hawaii), it is possible that the long days after the induction affect the rate of growth. Changes in growth could affect the inflorescence development and consequently promote the death of the shoots.

6.5. Conclusions

The stress that affects the flowering of *H. rostrata* by promoting the apex death might have different origin or the factors studied act in a complex fashion. Temperature seems not to be a primary factor inducing the death. The results of the last experiment showed that there were no differences among floral, vegetative and dead shoots due shoot density for the first two generations. These results contrast with the results from experiments (4.3.1 and 6.3.1) where more floral and fewer dead shoots were observed at low shoot density. However, the plants were at different stages of growth when subjected to induction. The stage of the shoots being induced and the synchronization of their growth may be an important trigger of apex death.

It was clear that the flowering and death of shoots was differentially affected with the generation, since the highest percentages of floral, vegetative, and dead shoots occurred in the second, third, and first generations, respectively.

Since apex death could occur during induction and/or during the flowers differentiation the causes could be different. The limited availability of carbohydrate and the increased competition between shoots in the clump and/or among flowers in the inflorescence were suggested as the alternative causes of dead.

CHAPTER 7

GENERAL CONCLUSIONS

From these experiments it possible conclude that:

Seasonal blooming of *H. rostrata* from March to July in Hawaii is due to this species requirement for short days to trigger the transition of the apex from vegetative to reproductive. If shoots were not under inductive short days the terminal apex would produce more leaves even if it had reached the competent stage to be induced. Each shoot bears a variable number of leaves, subtending the inflorescence, which depends on the number present at induction. The shoots are competent to be induced when they had three or more leaves.

Inflorescence induction, initiation and development takes a long period (at least 21 weeks) and occurs without external evidence of these processes until the inflorescence emerges from the pseudostem. The more obvious morphological changes at the apex during the transition are changes in primordium form (bract formation), bract primordia number, and the development of axillary bud primordia (which gives origin to flowers) closer to the meristem. Reproductive status was easily determined under the microscope when at least three bracts and the first axillary bud were observed. Flower differentiation on the cincinnus begins when many bracts are well developed. The inflorescence will emerge from the pseudostem from 8 to10 weeks after flower primordia differentiation.

H. rostrata can be described as a qualitative short day plant. The critical daylength is between 11h 45m and 12h. Imposing short daylengths for 4 or more weeks

can induce flowering, and the number of shoots within a plant induced increases with the number of weeks under inductive short days. The inflorescences began to emerge 21 weeks after the start of inductive short days.

Flowering can be inhibited using 4 hours of light from incandescent bulbs (1.9 Wm^{-2}) as supplemental light to the natural daylength or night-break. Both methods of flower inhibition can be used to extend the vegetative phase of growth in order to manipulate the blooming in this species.

Night temperatures between 16 and 26 °C for 4 to 8 weeks were not a flower inductive factor when the daylength was longer than the critical daylength. However, lower night temperature during induction increased the sensitivity of shoots to be induced to flower.

The high number of dead shoot apices affected the yield of inflorescences. Apex death was related to shoot density and shoot generation. The death of shoots was affected by generation, with the highest percentage of dead shoots occurring in the first generation after planting. The developmental stage of induced shoots and the synchronization of their growth could be factors promoting apex death. Since apex death could occur during induction and/or during flower differentiation, the trigger might have different origins. Competition between shoots in the clump and/or among flowers in the inflorescence is a possible explanation for the cessation of apex development and death of the growing point as has been shown for rice and other grasses. In these crops, availability of carbohydrate, light intensity and/or temperature-mediated respiration have been associated with apex death. .

These studies show that flower production of *H. rostrata* off-season is possible by manipulating flower induction with the use of short days and inhibition of flowering with extended daylength or night-break lighting. While that plant density and competition are likely factors to be considered during production since they are related with shoot apex death.

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APPENDIX
(TABLES)

Table IV.i. ANOVA Effect of daylength and number of weeks under treatment on floral shoots percentage in *H. rostrata*. Data transformed by ArcSin$\sqrt{x}$.

Source	df	Mean-Square	F-ratio	P
Daylength	2	99.015	0.870	0.423
Weeks	4	222.784	1.957	0.110
Daylength x Weeks	8	83.544	0.734	0.661
Error	75	113.867		

Table V.ii. ANOVA of regressing number of leaves at onset of treatments on the number of leaves at flowering in *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Leaves at flowering	1	321.186	404.367	0.0001
Error	122	96.903	0.794	

Table IV.iii. ANOVA Effect of daylength and number of weeks under treatment on vegetative shoots in *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	2	0.100	0.045	0.956
Weeks	4	0.294	0.133	0.970
Daylength x Weeks	8	1.294	0.582	0.789
Error	75	2.222		

Table IV.iv. ANOVA Effect of daylength and number of weeks under treatment on death shoots in *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	2	3.900	2.034	0.138
Weeks	4	1.739	0.907	0.465
Daylength x Weeks	8	0.789	0.411	0.911
Error	75	1.918		

Table IV.v. ANOVA Effect of daylength and temperature on number of total shoots in *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	2	11.431	4.236	0.019
Temperature	2	25.347	9.393	0.000
Daylength x Temperature	4	0.931	0.345	0.847
Error	63	2.698		

Table IV.vi. ANOVA Effect of daylength and temperature on number of shoots with potential to flower in *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	2	0.264	0.390	0.679
Temperature	2	2.514	3.716	0.030
Daylength x Temperature	4	0.285	0.421	0.793
Error	63	0677		

Table IV.vii. ANOVA Effect of daylength and temperature on number of inflorescences per pot in *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	2	0.889	5.895	0.005
Temperature	2	0.347	2.303	0.108
Daylength x Temperature	4	0.118	0.783	0.541
Error	63	0.151		

Table IV.viii. ANOVA Effect of daylength and temperature on inflorescences per pot in *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	2	0.889	5.895	0.005
Temperature	2	0.347	2.303	0.108
Daylength x Temperature	4	0.118	0.783	0.541
Error	63	0.151		

Table IV.ix. ANOVA Effect of daylength and temperature on vegetative shoots per pot in *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	2	0.389	0.398	0.674
Temperature	2	1.097	1.122	0.332
Daylength x Temperature	4	0.347	0.355	0.840
Error	63	0.978		

Table IV.x. ANOVA Effect of daylength and temperature on death shoots per pot in *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	2	0.931	1.274	0.287
Temperature	2	1.389	1.902	0.158
Daylength x Temperature	4	0.285	0.390	0.815
Error	63	0.730		

Table IV.xi. ANOVA Effect of daylength and temperature on vegetative shoots developed after treatment in *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	2	10.722	6.721	0.002
Temperature	2	14.889	9.333	0.000
Daylength x Temperature	4	0.681	0.427	0.789
Error	63	1.595		

Table IV.xii. ANOVA Effect of daylength and temperature on total of vegetative shoots in *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	2	15.167	5.189	0.008
Temperature	2	23.042	7.884	0.001
Daylength x Temperature	4	1.083	0.371	0.829
Error	63	2.923		

Table V.i. ANOVA Effect of supplemental daylength on the total of shoots of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	1	1.563	0.342	0.568
Error	14	4.562		

Table V.ii. ANOVA Effect of supplemental daylength during short days on vegetative shoots of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	1	0.562	0.243	0.630
Error	14	2.313		

Table V.iii. ANOVA Effect of supplemental daylength during short days on floral shoots of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	1	0.250	0.259	0.619
Error	14	0.964		

Table V.iv. ANOVA Effect of supplemental daylength during short days on dead shoots of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	1	0.562	0.232	0.637
Error	14	2.420		

Table V.v. ANOVA Effect of supplemental daylength during short days on the number of leaves per shoot of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	1	22.344	8.913	0.011
Error	13	2.507		

Table V.vi. ANOVA Effect of natural short-day, night-break and supplemental-light on floral shoots of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	2	2.778	16.176	0.000
Error	33	0.172		

Table V.vii. ANOVA Effect of natural short-day, night-break and supplemental-light on vegetative shoots of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	2	4.778	6.045	0.006
Error	33	0.790		

Table V.viii. ANOVA Effect of natural short-day, night-break and supplemental-light on death shoots of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	2	0.528	0.995	0.380
Error	33	0.530		

Table V.ix. ANOVA Effect of natural short-day, night-break and supplemental-light on total shoot number of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	2	1.361	1.098	0.345
Error	33	1.240		

Table V.x. ANOVA Effect of sequence extended-natural long days on total shoot number of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	1	0.250	0.070	0.795
Error	14	3.554		

Table V.xi. ANOVA Effect of sequence extended-natural long days on the number leaves at the onset of treatment of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	1	0.063	0.159	0.697
Error	14	0.394		

Table V.xii. ANOVA Effect of sequence extended-natural long days on the number leaves of *H. rostrata* at the end of the experiment.

Source	df	Mean-Square	F-ratio	P
Daylength	1	0.111	0.137	0.717
Error	14	0.808		

Table V.xiii. ANOVA Effect of sequence extended-natural long days on pseudostem height of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	1	927.202	2.353	0.147
Error	14	394.100		

Table V.xiv. ANOVA Effect of sequence extended-natural long days on apex location above the soil of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	1	569.300	1.520	0.238
Error	14	374.532		

Table VI.i. ANOVA Effect of density of shoots and leaf tearing on floral shoots of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	8.167	20.417	0.000
Tearing	1	0.167	0.417	0.526
Density x Tearing	1	0.167	0.417	0.526
Error	20	0.400		

Table VI.ii. ANOVA Effect of density of shoots and leaf tearing on vegetative shoots of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	16.667	10.989	0.003
Tearing	1	1.500	0.989	0.332
Density x Tearing	1	1.500	0.989	0.332
Error	20	1.517		

Table VI.iii. ANOVA Effect of density of shoots and leaf tearing on death shoots of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	104.167	29.343	0.000
Tearing	1	2.667	0.751	0.396
Density x Tearing	1	1.500	0.423	0.523
Error	20	3.550		

Table VI.iv. ANOVA Effect of density of shoots, daylength, and leaf pruning on number of floral shoots of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	2	437.408	1.088	0.341
Daylength	1	55814.859	138.805	0.000
Pruning	1	118.908	0.296	0.588
DensityxDaylength	2	112.310	0.279	0.757
DensityxPruning	2	783.211	1.948	0.148
DaylengthxPruning	1	116.730	0.290	0.591
DensityxDaylengthxPruning	2	45.880	0.114	0.892
Error	108	402.110		

Table VI.v. ANOVA Effect of density of shoots, daylength, and leaf pruning on number of vegetative shoots of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	2	494.693	1.088	0.341
Daylength	1	6874.797	22.643	0.000
Pruning	1	28.908	0.095	0.758
DensityxDaylength	2	764.546	2.518	0.085
DensityxPruning	2	131.663	0.434	0.649
DaylengthxPruning	1	68.328	0.225	0.636
DensityxDaylengthxPruning	2	127.604	0.420	0.658
Error	108	303.619		

Table VI.vi. ANOVA Effect of density of shoots, daylength, and leaf pruning on number of death shoots of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	2	83.023	0.178	0.837
Daylength	1	20583.797	44.193	0.000
Pruning	1	153.383	0.329	0.567
DensityxDaylength	2	256.703	0.551	0.578
DensityxPruning	2	1217.256	2.613	0.078
DaylengthxPruning	1	230.868	0.496	0.483
DensityxDaylengthxPruning	2	896.981	1.926	0.151
Error	108	465.775		

Table VI.vii. ANOVA Effect of daylength on floral shoots for the first generation of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	1	5.208	34.117	0.000
Error	118	0.153		

Table VI.viii. ANOVA Effect of daylength on floral shoots for the second generation of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	1	8.524	73.746	0.000
Error	118	0.116		

Table VI.ix. ANOVA Effect of daylength on floral shoots for the third generation of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	1	3.099	49.564	0.000
Error	118	0.063		

Table VI.x. ANOVA Effect of daylength on vegetative shoots for the first generation of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	1	0.093	4.504	0.036
Error	118	0.021		

Table VI.xi. ANOVA Effect of daylength on vegetative shoots for the second generation of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	1	2.494	25.622	0.000
Error	118	0.097		

Table VI.xii. ANOVA Effect of daylength on vegetative shoots for the third generation of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	1	0.507	4.675	0.033
Error	118	0.108		

Table VI.xiii. ANOVA Effect of daylength on death shoots for the first generation of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	1	3.912	23.022	0.000
Error	118	0.170		

Table VI.xiv. ANOVA Effect of daylength on death shoots for the second generation of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	1	1.797	17.320	0.000
Error	118	0.104		

Table VI.xv. ANOVA Effect of daylength on death shoots for the third generation of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Daylength	1	0.313	5.508	0.005
Error	118	0.057		

Table VI.xvi. ANOVA Effect of density of shoots, daylength, and leaf pruning on floral shoots from the first generation for group 1 and 2 of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	0.089	0.526	0.471
Daylength	1	4.356	25.775	0.000
Pruning	1	0.089	0.526	0.471
DensityxDaylength	1	0.139	0.822	0.368
DensityxPruning	1	0.272	1.611	0.208
DaylengthxPruning	1	0.356	2.104	0.151
DensityxDaylengthxPruning	1	0.139	0.822	0.368
Error	72	0.169		

Table VI.xvii. ANOVA Effect of density of shoots, daylength, and leaf pruning on floral shoots from the second generation for group 1 and 2 of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	0.143	1.010	0.318
Daylength	1	5.805	40.988	0.000
Pruning	1	0.000	0.001	0.972
DensityxDaylength	1	0.030	0.212	0.647
DensityxPruning	1	0.000	0.001	0.972
DaylengthxPruning	1	0.014	0.097	0.756
DensityxDaylengthxPruning	1	0.014	0.097	0.756
Error	72	0.142		

Table VI.xviii. ANOVA Effect of density of shoots, daylength, and leaf pruning on floral shoots from the third generation for group 1 and 2 of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	0.006	0.083	0.775
Daylength	1	2.206	28.391	0.000
Pruning	1	0.024	0.308	0.581
DensityxDaylength	1	0.006	0.083	0.775
DensityxPruning	1	0.005	0.061	0.805
DaylengthxPruning	1	0.024	0.308	0.581
DensityxDaylengthxPruning	1	0.005	0.061	0.805
Error	72	0.078		

Table VI.xix. ANOVA Effect of density of shoots, daylength, and leaf pruning on vegetative shoots from the first generation for group 1 and 2 of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	0.006	0.173	0.697
Daylength	1	0.139	4.317	0.041
Pruning	1	0.001	0.043	0.836
DensityxDaylength	1	0.006	0.173	0.679
DensityxPruning	1	0.001	0.043	0.836
DaylengthxPruning	1	0.001	0.043	0.836
DensityxDaylengthxPruning	1	0.001	0.043	0.836
Error	72	0.032		

Table VI.xx. ANOVA Effect of density of shoots, daylength, and leaf pruning on vegetative shoots from the second generation for group 1 and 2 of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	0.116	1.052	0.309
Daylength	1	2.021	18.280	0.000
Pruning	1	0.033	0.295	0.588
DensityxDaylength	1	0.021	0.186	0.667
DensityxPruning	1	0.164	1.479	0.228
DaylengthxPruning	1	0.143	1.294	0.259
DensityxDaylengthxPruning	1	0.086	0.774	0.382
Error	72	0.111		

Table VI.xxi. ANOVA Effect of density of shoots, daylength, and leaf pruning on vegetative shoots from the third generation for group 1 and 2 of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	0.669	6.203	0.015
Daylength	1	0.423	3.920	0.052
Pruning	1	0.038	0.355	0.553
DensityxDaylength	1	0.478	4.430	0.039
DensityxPruning	1	0.063	0.587	0.446
DaylengthxPruning	1	0.040	0.369	0.546
DensityxDaylengthxPruning	1	0.040	0.369	0.546
Error	72	0.108		

Table VI.xxii. ANOVA Effect of density of shoots, daylength, and leaf pruning on death shoots from the first generation for group 1 and 2 of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	0.009	0.044	0.835
Daylength	1	2.509	12.619	0.001
Pruning	1	0.059	0.295	0.589
DensityxDaylength	1	0.184	0.924	0.340
DensityxPruning	1	0.217	1.092	0.300
DaylengthxPruning	1	0.334	1.678	0.199
DensityxDaylengthxPruning	1	0.125	0.631	0.430
Error	72	0.199		

Table VI.xxiii. ANOVA Effect of density of shoots, daylength, and leaf pruning on death shoots from the second generation for group 1 and 2 of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	0.023	0.177	0.675
Daylength	1	0.738	5.725	0.019
Pruning	1	0.067	0.067	0.473
DensityxDaylength	1	0.035	0.035	0.620
DensityxPruning	1	0.233	0.002	0.183
DaylengthxPruning	1	0.002	0.399	0.914
DensityxDaylengthxPruning	1	0.399	0.129	0.083
Error	72	0.129		

Table VI.xxiv. ANOVA Effect of density of shoots, daylength, and leaf pruning on death shoots from the third generation for group 1 and 2 of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	0.478	15.144	0.000
Daylength	1	0.494	15.637	0.000
Pruning	1	0.012	0.383	0.538
DensityxDaylength	1	0.229	7.267	0.009
DensityxPruning	1	0.111	3.525	0.064
DaylengthxPruning	1	0.015	0.465	0.498
DensityxDaylengthxPruning	1	0.119	3.766	0.056
Error	72	0.032		

Table VI.xxv. ANOVA Effect of density of shoots, daylength, and leaf pruning on floral shoots from the first generation for group 2 and 3 of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	0.001	0.010	0.922
Daylength	1	2.689	18.766	0.000
Pruning	1	0.022	0.155	0.695
DensityxDaylength	1	0.006	0.039	0.844
DensityxPruning	1	0.939	6.553	0.013
DaylengthxPruning	1	0.501	3.499	0.065
DensityxDaylengthxPruning	1	0.068	0.475	0.493
Error	72	0.143		

Table VI.xxvi. ANOVA Effect of density of shoots, daylength, and leaf pruning on floral shoots from the second generation for group 2 and 3 of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	0.362	4.619	0.035
Daylength	1	6.356	81.040	0.000
Pruning	1	0.121	1.548	0.217
DensityxDaylength	1	0.004	0.048	0.827
DensityxPruning	1	0.104	1.325	0.254
DaylengthxPruning	1	0.014	0.176	0.676
DensityxDaylengthxPruning	1	0.014	0.176	0.676
Error	72	0.078		

TableVI. xxvii. ANOVA Effect of density of shoots, daylength, and leaf pruning on floral shoots from the third generation for group 2 and 3 of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	0.001	0.026	0.872
Daylength	1	1.886	49.498	0.000
Pruning	1	0.024	0.628	0.431
DensityxDaylength	1	0.001	0.026	0.872
DensityxPruning	1	0.005	0.125	0.725
DaylengthxPruning	1	0.024	0.628	0.431
DensityxDaylengthxPruning	1	0.005	0.125	0.725
Error	72	0.038		

Table VI.xxviii. ANOVA Effect of density of shoots, daylength, and leaf pruning on vegetative shoots from the first generation for group 2 and 3 of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	0.022	3.097	0.083
Daylength	1	0.022	3.097	0.083
Pruning	1	0.001	0.194	0.661
DensityxDaylength	1	0.022	3.097	0.083
DensityxPruning	1	0.001	0.194	0.661
DaylengthxPruning	1	0.001	0.194	0.661
DensityxDaylengthxPruning	1	0.001	0.194	0.661
Error	72	0.007		

Table VI.xxix. ANOVA Effect of density of shoots, daylength, and leaf pruning on vegetative shoots from the second generation for group 2 and 3 of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	0.319	4.490	0.038
Daylength	1	1.180	16.623	0.000
Pruning	1	0.394	5.554	0.021
DensityxDaylength	1	0.037	0.519	0.474
DensityxPruning	1	0.002	0.026	0.873
DaylengthxPruning	1	0.002	0.026	0.873
DensityxDaylengthxPruning	1	0.002	0.026	0.873
Error	72	0.071		

Table VI.xxx. ANOVA Effect of density of shoots, daylength, and leaf pruning on vegetative shoots from the third generation for group 2 and 3 of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	0.233	2.554	0.014
Daylength	1	0.008	0.091	0.763
Pruning	1	0.095	1.036	0.312
DensityxDaylength	1	0.018	0.192	0.663
DensityxPruning	1	0.132	1.448	0.233
DaylengthxPruning	1	0.008	0.084	0.773
DensityxDaylengthxPruning	1	0.097	1.062	0.306
Error	72	0.091		

Table VI.xxxi. ANOVA Effect of density of shoots, daylength, and leaf pruning on death shoots from the first generation for group 2 and 3 of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	0.000	0.002	0.963
Daylength	1	1.850	11.521	0.001
Pruning	1	0.042	0.262	0.611
DensityxDaylength	1	0.042	0.262	0.611
DensityxPruning	1	0.834	5.191	0.026
DaylengthxPruning	1	0.475	2.960	0.090
DensityxDaylengthxPruning	1	0.059	0.365	0.547
Error	72	0.161		

Table VI.xxxii. ANOVA Effect of density of shoots, daylength, and leaf pruning on death shoots from the second generation for group 2 and 3 of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	0.023	0.326	0.570
Daylength	1	1.706	24.424	0.000
Pruning	1	0.138	1.968	0.165
DensityxDaylength	1	0.067	0.960	0.330
DensityxPruning	1	0.138	1.968	0.165
DaylengthxPruning	1	0.088	1.257	0.266
DensityxDaylengthxPruning	1	0.088	1.257	0.266
Error	72	0.070		

Table VI.xxxiii. ANOVA Effect of density of shoots, daylength, and leaf pruning on death shoots from the third generation for group 2 and 3 of *H. rostrata*.

Source	df	Mean-Square	F-ratio	P
Density	1	0.060	1.094	0.299
Daylength	1	1.322	24.265	0.000
Pruning	1	0.000	0.000	0.994
DensityxDaylength	1	0.001	0.018	0.892
DensityxPruning	1	0.198	3.641	0.060
DaylengthxPruning	1	0.000	0.002	0.968
DensityxDaylengthxPruning	1	0.208	3.826	0.054
Error	72	0.054		